Pan-American Society for Evolutionary Developmental Biology

Inaugural 2015 Meeting

Berkeley, CA *August 5-9, 2015*

Welcome from the President

On behalf of the Council, I would like to say, “Welcome, Bienvenue, Bienvenida, and Bem-vindo” to all participants of the Inaugural meeting of the *Pan-American Society for Evolutionary Developmental Biology*!

For the first time, over 300 members of our new Society will sit in a single room for 3 days to discuss the past accomplishments and future challenges of our field. To spark this discussion, we have organized a diverse and exciting line-up of speakers and poster presentations from around the world (including my own poster presentation!), and whether you are a student, postdoc, junior or senior faculty, you have a vital role to play at this inaugural meeting – all of our approaches, experiences, and perspectives are collectively needed to help understand the goals and questions of our field and help define the common core of evo-devo. Future meetings of the Society will likely adopt a more open format for speakers with parallel sessions, so please take full advantage of this inaugural meeting to openly exchange ideas across the barriers imposed by the sub disciplines of our field. Lets not forget the other exciting first at this inaugural meeting – our recognition and celebration of pioneers in the field of evo-devo, as well as our highly promising students, postdocs, and early career researchers.

From its conception to its birth, establishment of the *Pan-American Society for Evolutionary Developmental Biology* and the organization of its inaugural meeting presented us with many challenges over the past 2 years. It was the hard work and dedication of the founding members of the Council and Organizers, Nipam H. Patel and Christopher J. Lowe, which made the Society and Inaugural meeting happen. For this, I am deeply grateful. Observing their remarkable dedication and determination over the last 2 years, and the result, is what makes this a truly historical event for our field in the Americas. I end by thanking our sponsors for their generous support of all the different events at this meeting.

I wish you all an inspiring conference,

Ehab Abouheif
President
Pan-American Society for Evolutionary Developmental Biology

Organizing Committee
Nipam H. Patel
Christopher J. Lowe
Karen E. Sears
Ehab Abouheif

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Local Organizers
Nipam H. Patel
Christopher J. Lowe
Karen D. Crow
Chelsea D. Specht

Cover credit:
Nipam Patel, Ariel Pani, Maggie Rigney, Erin Jarvis Alberstat, and Ryan Earley

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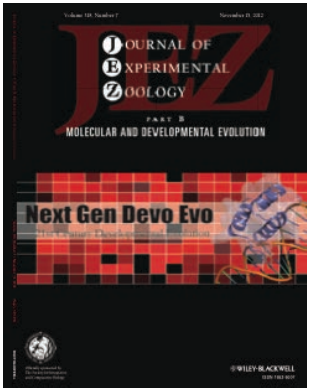
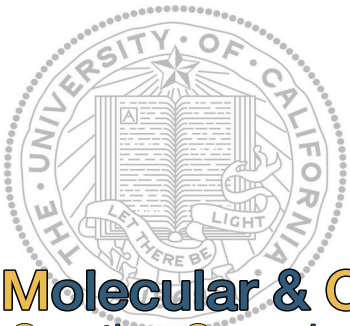
Pan-American Society for Evolutionary Developmental Biology

Evo-Devo-Eco Network



Journal of Experimental Zoology B (JEZB): Molecular Developmental Evolution

Society for Developmental Biology



UC Berkeley: Molecular & Cell Biology
Genetics, Genomics & Development

Molecular & Cell Biology
Genetics, Genomics & Development

General Information

Address: Clark Kerr Campus, 2601 Warring St., Berkeley, CA, Tel# 510-642-6290

Maps: Berkeley area and the Clark Kerr campus maps are on pages 149 and 150 at the end of this Meeting Program.

Meals: Meals begin with breakfast on Thursday Aug. 6th and end with breakfast on Sunday Aug. 9th. Breakfast starts at 7 AM. Lunch goes from Noon to 1:30 PM and dinner from 6:00 to 7:30 PM. All meals are in the dining center located in Building #10. To avoid congestion and long lines, we ask that some of you hold back (visit the posters, talk to friends) until 12:30PM and 6:30PM. There are tables indoors and outdoors and you can even sit on the grass near the dining center.

WiFi Access: WiFi will be available free of charge throughout the Clark Kerr campus including residence halls.

Lectures: All plenary lectures will be in the Krutch Theater (Building #14). Concurrent Sessions will be held in both the Krutch Theater and Garden Room on the morning of Friday Aug. 7th. The Garden Room is located in Building #10 near the dining center and it only takes a minute or two to walk between the two venues.

Posters: The poster sessions will be held in the Ginko Courtyard. Presenters should place their posters in the numbered positions anytime before lunch on Thursday Aug. 6th and take them down by dinnertime on Saturday Aug. 8th. There is sufficient room for all posters to stay up for the entire meeting. If you are worried about leaving your poster out at night, you can also take them down in the evening and put them back up before each poster session.

Information for Plenary Speakers: Please come to hook up your laptop at least 30 minutes prior to your session to make sure that everything is running correctly. Volunteers and the session chairs will be on hand to assist you. We should be able to hook up to four laptops at a time into the system, in order to transition between speakers quickly. Remember that you have 25 minutes for your talk and 5 minutes for questions (the Keynote speakers will have 35 minutes plus 10 minutes for questions).

Information for Concurrent Session Speakers: Please come to the session at least 30 minutes in advance to make sure your presentation is ready. We hope to consolidate six presentations onto four laptops or fewer so that we do not have to disconnect and connect devices during a session. Remember that you have 12 minutes for your talk and 3 minutes for questions. It is critical that we stick to the schedule so that people can move back and forth between the two lecture venues if they wish to do so.

time	Wednesday (Aug 5th)	Thursday (Aug 6th)	Friday (Aug 7th)	Saturday (Aug 8th)
7:00 - 8:00 AM		Breakfast	Breakfast	Breakfast
8:00 - 8:30 AM	*8AM - 3PM*	Mark Martindale		
8:30 - 9:00 AM	*Tribolium Meeting*	Rich Palmer	Concurrent Sessions	Robb Krumlauf
9:00 - 9:30 AM	*Rm 104*	Jocelyn Hall	2 X 6 talks (15 min)	James Umen
9:30 - 10:00 AM		Veronica Hinman	see next page	Manu Prakash
10:00 - 10:30 AM		Coffee Break	Coffee Break	Coffee Break
10:30 - 11:00 AM		Craig Miller	Concurrent Sessions	Deniz Erezylimaz
11:00 - 11:30 AM		Chris Amemiya	2 X 6 talks (15 min)	Alexa Bely
11:30 AM - Noon		Mansi Srivastava	see next page	Bob Reed
Noon - 12:30 PM		Lunch	Lunch	Lunch
12:30 - 1:00 PM		Noon - 1:30 PM	Noon - 1:30 PM	Noon - 1:30 PM
1:00 - 1:30 PM				
1:30 - 2:00 PM		Posters	Tamara Franz Odendaal	Matt Rockman
2:00 - 2:30 PM	2 PM – 6 PM, Check-In		Julia Bowsher	Stacey Smith
2:30 - 3:00 PM	& Registration	(refreshments)	Posters & refreshments	Posters & refreshments
3:00 - 3:30 PM				
3:30 - 4:00 PM	*3-6 PM*		Rachel Collin	Ralf Sommer
4:00 - 4:30 PM	*Council Meeting*		Frietson Galis	Vivian Irish
4:30 - 5:00 PM	Rm 102	Mark Rebeiz	Break	Break
5:00 - 5:30 PM		Jose Javier Neto	James Hanken	Matt Gibson
5:30 - 6:00 PM	Dinner on	Catherine Linnen	Angela Hay	Poster & Talk Awards
6:00 - 6:30 PM	your own	Dinner	Dinner	Dinner
6:30 - 7:00 PM		6:00 PM - 7:30 PM	6:00 PM - 7:30 PM	5:45 PM - 7:15 PM
7:00 - 7:30 PM	Introduction			
7:30 - 8:00 PM	Keynote talks:	Workshops	Igor Schneider	Award talks
8:00 - 8:30 PM	Sean Carroll	(concurrent in	Kim Cooper	Rudy Raff
8:30 - 9:00 PM	Neelima Sinha	Krutch Theater		Natalia Pabon-Mora
9:00 - 9:30 PM	Opening Reception	and Garden Room)	Future of EvoDevo	Closing Reception
9:30 - 10:00 PM				

Sunday Breakfast
7AM - 9AM
Departure by Noon

Concurrent session schedule (Friday Aug 7; 12 min talk + 3 min question

	Krutch Theater	**Garden Room**
8:30 - 8:45 AM	Andrew Gillis	Carolyn Wessinger
8:45 - 9:00 AM	Alberto Stolfi	Evangeline Ballerini
9:00 - 9:15 AM	Carlos Infante	Alma Pineyro-Nelson
9:15 - 9:30 AM	Jennifer Maier	Cecilia Zumajo Cardona
9:30 - 9:45 AM	Karen Crow	Marianna Benitez
9:45 - 10:00 AM	Ricardo Mallarino	Deirdre Lyons
Break 10:00 - 10:30 AM		
10:30 - 10:45 AM	Sylvain Marcelini	Erin Jarvis Alberstat
10:45 - 11:00 AM	Jessica Gray	Emily Delaney
11:00 - 11:15 AM	Brent Hawkins	Yi-Jyun Luo
11:15 - 11:30 AM	Yi-Hsien Su	Eric Camino
11:30 - 11:45 AM	Arnaud Martin	Pamela Windsor-Reid
11:45 AM - Noon	Paul Gonzalez	Sofia Casasa

Detailed Program
Map of Clark Kerr Campus on page 150 of this booklet

Wednesday August 5th

08.00-15.00 Tribolium Meeting (Preconference)
Room 104, Krutch Theater (Building 14)

14.00-18.00 Registration
Building 1

15.00-18.00 Council Meeting
Room 102, Building 14

18.00-19.00 Dinner on your own

19.00-19.30 Welcome & Introduction
Krutch Theater

Ehab Abouheif as President (McGill University)
Nipam Patel (University of California, Berkeley)
Chris Lowe (Stanford University)

19.30-21.00 Keynote Lectures
(*Chair: Ehab Abouheif, McGill University*)
Krutch Theater

19.30-20.15 Sean Carroll (University of Wisconsin-Madison & HHMI)
Gene Co-option and the Evolution of Novelties
The origins of novelties pose some of the most interesting yet experimentally difficult problems in evolutionary biology. Morphological novelties in animals are generally thought to arise through the co-option of regulatory genes, but how such changes arise has not been explored in depth. We have found that the co-option of the *wingless* gene and novel features of *wingless* gene expression in Drosophila arose from new activities that evolved within pre-existing regulatory sequences. We have recently begun to explore the origins of biochemical novelties, as exemplified by the complex mixtures of proteins in animal venoms. We have found that rattlesnake venom toxins have evolved via both gene co-option and gene duplication, and venom diversity has also entailed gene losses over relatively short timescales.

20.15-21.00 Neelima Sinha (University of California, Davis)
Gene network modules regulating natural diversity in leaf shape
The leaf is a key evolutionary innovation in the land plant lineage that allowed plants to efficiently capture solar energy for carbon fixation. A variety of leaf morphological and physiological traits have allowed adaptation to different natural

habitats, making the leaf a model organ for analyzing the mechanisms underlying natural morphological diversity in plants. However, the molecular mechanisms that give rise to leaf developmental variation are incompletely understood. The complexity of Gene Regulatory Networks leading to the formation of a mature leaf has precluded elucidation of the interspecific molecular dynamics in the leaf development. We performed comparative transcriptomics utilizing three *Solanum* species showing different leaf development characteristics. We utilized gene network construction to identify key network modules that play a role in leaf development. Super self-organizing map clustering, which can account for multiple factors by using a separate weighted layer for every factor, identifies major interspecific changes of gene expression patterns in leaf development; large interspecific differences in cell division/differentiation and peptidase activity, as well as subtle but significant interspecific differences in photosynthesis, translation and transcriptional regulation are seen. Our analyses suggest that not only massive differential gene expression but also changes in the system-level regulation of gene expression pattern differentiate leaf development between the species.

21.00-22.00 Opening Reception
Dining Center and Patio (Building 10)

Thursday August 6th

7.00-8.00 BREAKFAST
Dining Center

08.00-10.00 Plenary Talks Session 1
(*Chair: Billie Swalla, University of Washington*)
Krutch Theater

08.00-08.30 Mark Martindale (University of Florida)
Developmental constraints on the evolution of axial organization prior to the bilaterian explosion
Bilaterally symmetrical animals dominate extant life on the planet comprising over 99% of all described species. However, representatives of the earliest branching forms (sponges, placozoans, ctenophores and cnidarians) are still present and yet fail to display bilateral symmetry properties. Of particular relevance are the cnidarians (e.g. corals, anemones, and hydroids), since they are the agreed upon sister taxon to the Bilateria. Through experimental embryological and molecular evidence, I argue that changes in the patterns of gastrulation in the bilaterian ancestor allowed for a rapid expansion of distinct morphological features in descendent taxa. In particular, changes in the axial polarity of the position of endomesoderm formation, and resultant segregation of endomesoderm and neurogenic regions may have facilitated future novelties. I discuss possible molecular mechanisms to explain these changes, including the PAR system that plays important roles in polarizing the embryos of many bilaterian taxa. In the starlet sea anemone, *Nematostella vectensis* we show that PAR proteins are actively involved in the establishment of polarity in ectodermal epithelia at blastula stages, but are NOT asymmetrically localized in oocytes, eggs, or cleavage stages. Interestingly, PAR proteins are not expressed in the endomesodermal layer, which is itself a polarized epithelium. This suggests that the PAR system was involved in establishing epithelial patterning in early metazoans but the PAR system was co-opted by the bilaterian ancestor to early stages of development to polarize the primary embryonic axis.

08.30-09.00 Rich Palmer (University of Alberta)
Left-right asymmetry: The interplay between development, genetics and evolution
PART I) Natural selection eliminates variation from populations, generation after generation. So where does the new variation come from? Are mutation and recombination the primary sources of new variation (genes as leaders) or does the remarkable capacity of organisms to produce new forms as developmentally plastic responses to new environments -- at least initially -- also contribute (genes as followers)? A wide-

ranging survey of asymmetry variation within and among species of animals and plants offers some of the strongest evidence to date that a 'genes as followers' mode of evolution may be much more common than previously thought.
PART II) Development of bilaterian animals is often described as proceeding along three global (whole body) developmental axes: antero-posterior, dorso-ventral, and right-left. But differences in form between the sides of asymmetrical animals do not arise along a "right-left" gradient. Rather, they arise from a qualitative difference between how development proceeds along two medio-lateral axes — one on either side of the midplane. In dimorphic species that exhibit random asymmetry (right- and left-sided forms equally common) three questions arise: 1) Is direction of asymmetry (right- or left-sidedness) determined genetically? 2) Is development of right- or left-sidedness determined globally (whole-body level) or locally (individual organ-system level)? 3) Where right- or left-sidedness is not determined genetically, can direction of asymmetry be biased in one direction by environmental effects? Evidence from several crustacean taxa reveals intriguing relations between development, genetics and evolution of morphological asymmetries.

09.00-09.30 Jocelyn Hall (University of Alberta)

Basis of floral variation in Cleomaceae

Cleomaceae is a promising plant system to investigate the evolutionary developmental inquiries. The family exhibits substantial morphological variation and genomic resources are building. Moreover, it's sister relationship to Brassicaceae, which houses Arabidopsis, provides a powerful comparative framework for developing genetic hypotheses. We are broadly interested in the genetic basis for floral variation in the family with emphasis on floral symmetry, a trait of ecological significance due to its importance in plant-pollinator interactions. Most species of Cleomaceae are monosymmetric due to the perianth and reproductive whorls curving upwards. Some species have a large adaxial nectary gland, which impacts symmetry, and/or additional differences between top and bottom petal color and shape. Within the Cleomaceae similar mature morphologies across species are the result of different developmental pathways. We determined gene expression patterns in focal species with qRT-PCR and in situ hybridization. We focused our investigations on the role of the TCP transcription factor family in establishing floral monosymmetry within Cleomaceae. The TCP family has been recruited to establish monosymmetry in many taxa across angiosperms including Brassicaceae. We also conducted virus-induced gene silencing (VIGs) to characterize gene function. These experiments support the hypothesis that TCP contributes towards establishing monosymmetry in *C. violacea*, but intriguing patterns of B-class expression suggest these

genes need to be examined in more detail. We will compare these results to other members of Cleomaceae that exhibit a different developmental pathway to monosymmetry.

09.30-10.00 Veronica Hinman (Carnegie Mellon University)

Developmental Consequences of the Evolution of Transcription Factor Function

It is well documented that GRNs can evolve extensively through mutations to cis-regulatory modules. Transcription factor proteins that bind these cis-regulatory modules may also evolve to produce novelty. Coding changes, however, are considered to be more rare, because transcription factors are highly pleiotropic and hence are more constrained to evolve in ways that will not produce widespread detrimental effects. Recent technological advances have unearthed a surprising variation in DNA-binding abilities, such that individual transcription factors may recognize both a preferred primary motif and an additional secondary motif. This provides a source of modularity in function. In this talk, we will present recent work that shows that orthologous transcription factors can also evolve a changed preference for a lower affinity secondary binding motif, thereby offering an unexplored mechanism for GRN evolution. We demonstrate that this difference may allow for greater evolutionary change in timing of regulatory control and provide a mechanism through which organisms can evolve a changed response to signaling gradients. This uncovers a layer of transcription factor binding divergence that could exist for many pairs of orthologs.

10.00-10.30 COFFEE BREAK

10.30-12.00 Plenary Talks Session 2
(*Chair: Jeff Marcus, University of Manitoba*)

Krutch Theater

10.30-11.00 Craig Miller (University of California, Berkeley)

The beak of the fish: Developmental genetics of craniofacial evolution in sticklebacks

Understanding how changes in body pattern evolve remains a major goal of biology. Threespine stickleback fish provide a powerful system to address the developmental genetic bases of morphological evolution. Marine sticklebacks have repeatedly colonized and adapted to countless freshwater environments. To adapt to a diet of larger prey in freshwater, changes in craniofacial pattern evolve. Marine and freshwater fish can be crossed, allowing forward genetic dissection of evolved traits. Two craniofacial traits that evolve in freshwater are a reduction in gill raker number and an increase in pharyngeal tooth number. Gill raker reduction arises during embryogenesis, as increased spacing between periodic gill raker placodes. Evolved tooth gain arises late during larval

development, and is associated with an increased tooth replacement rate. Using genome-wide linkage mapping, we have mapped genomic regions controlling these two evolved traits. One genomic region with large effects on gill raker number contains *Fibroblast Growth Factor 20* (*Fgf20*), and induced mutations in *Fgf20* in marine fish phenocopy this evolved trait. The genomic region with the largest effect on evolved tooth gain contains a *cis*-regulatory allele of *Bone Morphogenetic Protein 6* (*Bmp6*). Induced mutations in a tooth enhancer and coding region of *Bmp6* result in opposite effects on tooth number, revealing complex roles of *Bmp* signaling in regulating tooth number. *Bmp6* coding region mutations are lethal when homozygous, while regulatory mutations are viable. Collectively these studies support the hypothesis that *cis*-regulatory alleles of pleiotropic developmental regulators are preferentially used in nature to evolve changes in body pattern.

11.00-11.30 Chris Amemiya (Benaroya Research Institute)

Genomics and evo-devo: Looking for novel stuff using comparative genomics approaches

Genome assemblies are being reported for large numbers of plant and animal species and thousands will be generated over the course of the next few years. Charles Darwin would no doubt revel in our ability to draw biological inferences from genome sequences. However, many genome papers submitted today are largely formulaic and descriptive, and the biological content in these reports is often embarrassingly low and wildly over-interpreted. Genomics can only take us so far without proper authentication via empirical biology. Here, I shall discuss some recent genome efforts in my lab whereby novel characteristics of the genomes can be exploited to learn about interesting and hitherto uninvestigated biological problems. My thesis is that, while comparisons of the “known” genetic components between species are important and necessary, many salient evo-devo insights will be gotten only when specifically looking for, and investigating, the differences. I will give a couple of examples, including one that suggests a new mechanism in the vertebrates for cellular and developmental programming based on the use of glycopolymers as mediators of biological function.

11.30-12.00 Mansi Srivastava (Harvard University)

Identifying essential molecular mechanisms for animal regeneration using a comparative approach

Most animal lineages have species with extensive regenerative capacity but it is unknown whether the underlying molecular mechanisms are shared by descent from a common regenerative ancestor, or whether they evolved independently. Since evolution tends to preserve core aspects of a process, comparing regeneration in distantly-related species can reveal

previously unknown mechanisms for regeneration. We developed the acoel worm, *Hofstenia miamia*, as a new model for studying regeneration. *Hofstenia* is distantly related to planarians, the well-established invertebrate model system. Studies of regeneration in *Hofstenia* revealed that mechanisms for patterning of new tissue during regeneration are very similar between acoels and planarians. This raises the possibility that other conserved mechanisms for regeneration can be found. High-throughput approaches combined with mechanistic studies in acoels and planarians are now allowing us to characterize the evolutionary history of many other aspects of regeneration

12.00-13.30 LUNCH
Dining Center

13.30-16.30 Poster Session (Posters P001-P179; see pages 50-143 of this booklet) & BREAK
Ginkgo Courtyard **Snacks and refreshments at 14:30**

16.30-18.00 Plenary Talks Session 3
(*Chair: Manfred Laubichler, Arizona State University & Santa Fe Institute*). This plenary session is sponsored by JEZB: Molecular Developmental Evolution.
Krutch Theater

16.30-17.00 Mark Rebeiz (University of Pittsburgh)
A circuit-based view of gene regulatory network co-option reveals unexpected paths for the evolution of novelty
The evolutionary origins of complex anatomical structures such as the eye or thumb remain a major puzzle in evolutionary developmental biology. The development of morphology is controlled by gene regulatory networks (GRNs) composed of transcription factors, signaling pathways, and the regulatory sequences (enhancers) they control to activate expression of structural genes that ultimately confer physical properties upon a tissue. Comparative expression analysis of macroevolutionary novelties such as the beetle's horn or turtle's shell indicate that network co-option is a major mechanism by which these structures evolve, as the genes deployed in these structures participate in networks used elsewhere during development. Here, we provide an example of network co-option underlying a recently evolved novelty in Drosophila melanogaster that we have traced to individual enhancers of an ancestral network. The posterior lobe is a recently evolved novel genital formation, unique to the D. melanogaster clade. By tracing the evolutionary history of an enhancer that drives posterior lobe expression of the Pox-neuro (Poxn) gene, we have discovered that this function likely evolved through the co-option of an ancestral element active in the embryonic posterior spiracles. Examination of several

additional genes of the Abd-B-regulated posterior spiracle network revealed expression of multiple network components during genital development, suggesting that this Hox-regulated network was partially co-opted during the origination of the posterior lobe structure. These findings provide a fine-scale picture of co-option, resolved to the level of regulatory connections, and illuminate how the ancestral network underlying a novelty may have unexpected beginnings.

17.00-17.30 Jose Xavier-Neto (LNBio - Brazilian National Biosciences Laboratory)

Cardiac fossilization is possible in an extinct basal teleost from the Cretaceous: Insights on the simplification of the outflow in ray-finned fish

Chambered hearts evolved from peristaltic pumps similar to those that equip most animal groups displaying circulatory pumps. The developmental mechanisms behind the evolutionary emergence of cardiac chambers remain unknown, but three hypotheses have been proposed (sequential, recruitment and patterning) and each has specific predictions that can be tested against ontogenetic, comparative and paleontological data. However, elucidating vertebrate cardiac evolution has been frustrated by lack of a cardiac fossil record. Thus, we set out to establish a proof of principle for cardiac fossilization. Here we demonstrate that cardiac preservation is possible in the extinct basic teleost fish *Rhacolepis buccalis* from the North-Eastern Brazilian Cretaceous. The cardiac fossils we found shed light on a century-old question on cardiac evo-devo in ray-finned fish, namely the evolutionary shift from a primitive valve-rich state to a derived, valve-depleted, condition of the teleost outflow tract. In contrast to extant teleosts, the *Rhacolepis buccalis* cardiac outflow tract displays a massive conus arteriosus with an intermediate number of valves between the dozens of basal ray-finned fish and the single valve of teleost such as the zebrafish. The *R. buccalis* outflow tract represents a morphological link between the multivalvar, conal, condition of basal gnathostomes and basal teleosts and the oligovalvar, bulbar, state of the outflow tract in higher teleosts. This snapshot of cardiac evolution indicates outflow tract divergence in actinopterygians was underway 119-113 Ma and shows that it is possible to scrutinize the fossil record for clues on cardiac structure and evolution.

17.30-18.00 Catherine Linnen (University of Kentucky)

From mutations to species: Causes and consequences of host-use variation in pine sawflies

To explain biological diversity, we must understand: (1) how genetic changes act through developmental mechanisms to alter phenotypes, (2) how these phenotypic changes impact the ability of organisms to survive and reproduce in nature, (3)

how natural selection and demographic processes shape this variation within and between populations, and (4) how these processes contribute to the formation of new species. Importantly, we must also determine the extent to which the answers to these questions are predictable/repeatable when examined across different organisms and traits. To address these questions, my lab uses insects in the genus *Neodiprion*, an experimentally tractable and phenotypically variable group of pine-feeding hymenopterans with convergent gains and losses of multiple traits. In this talk, I introduce this novel system and describe our ongoing efforts to develop genomic resources. Then, using host use as an example, I illustrate how we are identifying the proximate and ultimate mechanisms underlying phenotypic variation and assessing repeatability. Phenotypic, population genomic, and comparative data indicate that variation in needle width among pine species generates divergent selection on host-use traits within and between *Neodiprion* species; that divergent host use acts as a barrier to gene flow; and that changes in host use are associated with speciation. Ultimately, by combining recently developed molecular and genomic approaches with decades of natural history research, we hope to generate new insights into the origin and maintenance of phenotypic variation.

18.00-19.30 DINNER
Dining Center

19.30-22.00 Workshops- Five concurrent
Krutch Theater & Garden Room (Building 10)

1. Diversity in Evo-Devo and student mentor relationship
Billie Swalla (University of Washington)
Managing the mentor/student relationship; goals and expectations.
Annette Angus (University of California, Berkeley)
Fostering inclusion of underrepresented groups/people in Evo-Devo

2. Latin American challenges in Evo-Devo
Federico Brown (Universidade de São Paulo)
The main objective of this workshop is to identify issues hindering Evo-Devo research in Latin America, and to engage Society members and the research community at large in specific action plans. To accomplish this, workshop participants will discuss the current state of Evo-Devo in Latin America; five topics will be selected and discussed in small working groups to generate specific action plans for society members and workshop participants. Proposed topics for discussion include: (a) Inclusion of countries where Evo-Devo (or developmental biology) are not represented; (b) Collaborations and networking among groups; (c) Funding efforts; (d) Model systems for Evo-Devo and associated collecting or research permits;(e) Communicating and publishing EvoDevo in Latin America. Of course, these topics may be modified or new topics included in the agenda. Faculty of different Latin American

countries will moderate each discussion group. At the end we will have time for executive summary report and action plans. If you are interested in collaborating with laboratories, plan to conduct research, or are simply interested in promoting Evo Devo research in Latin America, please participate.

3. Evo-Devo Education Workshop

Trisha Wittkopp (University of Michigan)

Topics for discussion at this workshop will include: What are the strengths and challenges in evo-devo education right now? What resources can the society provide that would be helpful in your classrooms? Which textbooks are used most frequently? What would you want in an ideal textbook? Have you developed in-class activities that worked well for teaching evo-devo? When evo-devo is incorporated into a larger course (e.g., Introductory Biology), which key elements of the field should be covered?

4. Round Table Discussions on new and developing tools for emerging model systems in Evo-Devo.

Anastasios Pavlopoulos (Howard Hughes Medical Instittute)

Modern bioimaging and image analysis with light-sheet fluorescence microscopy

Yoshi Tomoyasu (Miami University) and David Angelini (Colby College)

Polishing up your RNAi techniques (mainly in insects)

Arnaud Martin, Nipam Patel & Jacob Corn (UC Berkeley)

CRISPR/Cas9 genome editing: Testing gene function in non-canonical model species

Rie Kusakabe (RIKEN)

Techniques for transient functional analyses of genes and cells

5. Discussion Panel on Theory in Evo Devo

Johannes Jaeger (Centre for Genomic Regulation (CRG)) & Manfred Laubichler (Arizona State University and Santa Fe Institute)

EvoDevo is facing a number of formidable challenges today. It needs to move on from the comparison of single traits or genes to systems level analyses of trait complexes and the underlying gene regulatory networks that generate them. It needs to shift focus from long-range comparisons between selected model organisms (confounded by convergent evolution and systems drift) to more fine-grained comparative analyses of developmental processes in more closely related non-model species. It needs to shift from qualitative descriptions to quantitative mechanisms of evolutionary change in developmental processes. All of these steps require novel systems-biology approaches based on quantitative measurement and data-driven mathematical modeling. In this panel discussion we review what has already been achieved in this direction, and seek to explore the potential and the possible pitfalls of these newly emerging approaches. Discussion topics include:

- what are the limits of current insights/models in EvoDevo?

- what do we want to understand? what is the appropriate level of explanation for developmental evolution?

- how general should such explanations be? is it possible to derive some kind of conceptual framework for systems EvoDevo?
- what kind of data are needed for an evolutionary systems biology? what techniques are there? in what kind of organisms?
- how can we understand the organization/structure of developmental processes?
- how do we model evolving developmental processes? what methods are available? which formalisms are useful/practical?
- why is it important to have dynamic rather than static, causal-mechanistic rather than statistical models?
- how should computational and experimental methods/people interact? how can we teach our students to make this happen?

Friday August 7th

7.00-8.30 BREAKFAST
Dining Center

08.30-10.00 Concurrent Talks Session Ai
(*Chair: Cassandra Extavour, Harvard University*)
Krutch Theater

8.30-8.45 Andrew Gillis (University of Cambridge)
Development and evolution of chondrichthyan gill arch appendages
Chondrichthyan fishes (sharks, skates, rays and holocephalans) are unique among extant jawed vertebrates in possessing appendages (branchial rays) that project laterally from the epi- and ceratobranchial cartilages of their gill arches. Over a century ago, Carl Gegenbaur proposed that pectoral appendages (i.e. paired fins, limbs) arose by transformation of a caudal gill arch, with the epi- and ceratobranchial cartilages giving rise to the girdle, and branchial rays giving rise to the fin/limb proper. I demonstrate that, unlike the endoskeleton of paired fins and limbs, chondrichthyan branchial rays derive from cranial neural crest cells. I also demonstrate that chondrichthyan branchial rays are patterned by a sonic hedgehog (Shh)-expressing epithelial signaling center that functions in both proliferative expansion and anteroposterior (AP) patterning of the gill arch endoskeleton: successively earlier loss of Shh signalling results in a progressive reduction of branchial ray number, while later loss of Shh signalling results in a reversal of branchial ray AP polarity. The branchial ray reduction phenotype is reminiscent of the progressive loss of digits seen with successively earlier loss of Shh function during tetrapod limb development, while the AP polarity phenotype is quite distinct from Shh loss-of-function phenotypes observed in paired fins and limbs. These experiments highlight branchial rays as a novel model for vertebrate paired appendage development, and as a tractable experimental embryological system with which to test classical hypotheses of serial homology relating to the origin of the jawed vertebrate body plan.

8.45-9.00 Alberto Stolfi (New York University)
Divergent mechanisms regulate conserved cardiopharyngeal and neural development in distantly related ascidians
Ascidians present a striking dichotomy between conserved phenotypes and divergent genomes: embryonic cell lineages and gene expression patterns appear unchanged even between species that share very little genome sequence similarity. We have sequenced the genomes of three Molgula species and adapted transgenesis techniques in order to

compare them directly to the distantly related model ascidian Ciona intestinalis. Comparisons between unequivocally homologous cell lineages at cellular resolution revealed extreme conservation of patterning and cell fate specification events in ascidian cardiopharyngeal and neural development, even in the morphologically divergent anural larvae of Molgula occulta. Although we found that the expression patterns of key developmental regulators are virtually identical among the Ascidiacea, cross-species transgenic assays uncovered incompatibility, or 'unintelligibility', of orthologous cis-regulatory sequences between Molgula and Ciona. In other words, these sequences drive identical expression patterns in their species of origin but fail to even remotely recapitulate this activity in cross-species assays. We show that this particularly acute manifestation of developmental system drift is likely due to changes in both cis- and trans-acting elements, hinting at widespread and frequent turnover of regulatory mechanisms underlying otherwise highly conserved aspects of ascidian embryogenesis.

9.00-9.15 Carlos Infante (University of Georgia)

Disentangling the regulatory network contributing to limb evolution in Anolis ectomorphs

The repeated radiation of Anolis lizards on the islands of the Greater Antilles has resulted in series of species, termed ecomorphs, with morphologies adapted to specific ecological niches on each island. We are interested in uncovering the developmental basis of limb length differences found among different ecomorphs. Previous work demonstrated that deletion of an enhancer for the Tbx4 gene can decrease limb length in mice. In a survey of orthologous DNA sequences in over 90 species of Caribbean anoles, we identified deletions in 3 of 7 short-limbed Anolis lineages, with no comparable deletions in long-limbed species. When we generated mouse knockins where the native mouse enhancer has been replaced with the enhancer from short-limbed or long-limbed species, there is a quantifiable increase in hindlimb bone size in long-limbed vs. short-limbed enhancer knockin mice. This demonstrates that the sequence deletion in this short-limbed Anolis alters enhancer function and may play an important role in the evolution of limb length in Anolis. To identify additional enhancers contributing to the evolution of Anolis ecomorphs, we have performed ChIP-Seq on embryonic tissues from Anolis carolinensis. By comparing ChIP-Seq signals across tissue types, we have identified active regulatory regions specific to the limbs. These limb-specific regions will be used to expand our survey of limb enhancers across Anolis ecomorphs to determine whether convergent ecomorph limb morphology is the result of similar or distinct alterations to the cis-regulatory network controlling limb development.

9.15-9.30 Jennifer Maier (University of Illinois at Urbana-Champagne)

Evolution of Hox gene expression and regulation in mammalian limbs

Indirect evidence from studies of mammalian limb evolution suggests that mammalian limb diversification has not occurred primarily by the evolution of new genes, but by differential regulation of existing genes shared by all mammals and inherited from an ancestral genetic toolkit. However, the specific genetic elements that regulate expression remain unknown for most genes, as does the way that these genetic elements have evolved to differentially regulate gene expression among species. This represents a major gap in our knowledge that critically undermines our ability to assess the impact of gene regulatory modification on morphological evolution. We seek to identify genetic elements that regulate limb development and may facilitate limb evolution in mammals. We used RNASeq to compare the transcriptomes of the developing limbs of several mammals, including mice, bats, opossums, and pigs. These studies identified significant differences in the expression levels of genes in the HoxA and HoxD clusters (9 genes) within and among the various species. To confirm this differential expression, we performed WISH. WISH generally confirmed the RNASeq results, and uncovered key differences in expression domains as well. We then used computational approaches to identify candidate enhancers for the HoxA and HoxD clusters, and functionally tested candidate enhancers using in vitro luciferase assays. Through this approach we identified several candidate enhancers with the potential to drive lineage-specific Hox expression patterns. We are currently testing the ability of these candidate enhancers to generate lineage-specific expression patterns and morphologies in vivo by creating transgenic mice.

9.30-9.45 Karen Crow (San Francisco State University)

There is more to the Hox Code than you thought. The "Distal Phase" HoxA/D expression pattern is an ancient module that is deployed in a variety of novel features in vertebrates

Fins and limbs are homologous structures patterned by a shared genetic repertoire of HoxA/D expression, or "the Hox limb building toolkit". A unique inversion of the HoxD expression pattern is associated with the most well characterized example of a novel fin/limb modification to date- the tetrapod autopod, where an inverted collinear HoxD expression pattern specifies digit identity and the origin of the thumb. This pattern also occurs in paddlefish pectoral fins and catshark paired fins, indicating that it arose in the common ancestor of jawed vertebrates. We refer to this pattern as 'distal phase' (DP) expression because it occurs in distal structures and is regulated independently. We argue that it may be deployed in a modular fashion, suggesting a greater

role in the evolution of morphological diversity in vertebrates than previously recognized. We demonstrated the first evidence for HoxD DP expression in a body plan feature beyond fins and limbs- the paddlefish barbel, and the first evidence for HoxA DP expression in the developing hindgut and vent of ray-finned fishes, suggesting that the limb-building program may have an expanded repertoire. Interestingly, HoxA DP expression is predicted by similar conformational properties between the HoxA/D cis-regulatory landscapes in zebrafish and mice, but has not been reported in vertebrate paired appendages. However, we found evidence for HoxA DP expression in claspers-modified pelvic fin structures in male cartilaginous fishes. Taken together, these data support the modularity of DP Hox expression pattern, and a greater role for the Hox code in evolution of novel body plan features.

9.45-10.00 Ricardo Mallarino (Harvard University)

How the mouse got its stripes: The molecular basis of stripe pattern formation in rodents

Mammalian color patterns, from zebra stripes to leopard spots, are among the most conspicuous characters in nature and have a profound impact in fitness; however, little is known about the mechanisms underlying their formation and evolution. Here we take advantage of the naturally occurring coat pattern in Rhabdomys, a rodent that evolved black and white longitudinal dorsal stripes, to investigate the mechanisms responsible for periodic patterning. In Rhabdomys, stripes form during development and differ in melanin deposition, with no difference in cell type or tissue architecture. An RNAseq screen along different dorsal regions of individuals from late embryogenic/early postnatal stages identified Alx3, a homeoprotein transcription factor, as a strong candidate specifying stripe formation. In Mus, Alx3 is expressed during mid development in skeletal tissues whereas in Rhabdomys, in addition to the skeleton, it is expressed in a new spatial domain along a subset of the dorsal skin that coincides with the region where stripes will form. Later in development, Alx3 expression remains patterned in the dermis, epidermis and developing hair follicles. In vivo functional tests using ultrasound-assisted lentiviral infections targeting different cell types revealed that Alx3 alters the expression of melanogenic enzymes and ChIPseq experiments are elucidating the mechanisms by which this occurs. In addition, Alx3 is differentially expressed along the dorsal stripes of chipmunks, which have independently evolved a similar striping pattern to Rhabdomys. Together, our results suggest that Alx3 is involved in patterning the longitudinal stripes in Rhabdomys and its function may be conserved across striped rodents.

08.30-10.00 Concurrent Talks Session Aii
(*Chair: Federico Brown,* Universidade de São Paulo)

Garden Room

8.30-8.45 Carolyn Wessinger (University of Kansas)
Parallel evolutionary shifts from bee to hummingbird pollination in *Penstemon* may be genetically easy (but difficult to reverse?)
Parallel evolution suggests certain phenotypic transitions are repeatedly favored, but also implies they can readily be generated through mutations to the underlying developmental genetic pathway. The wildflower genus Penstemon has experienced parallel shifts from bee to hummingbird pollination syndrome. This evolutionary transition involves shifts in flower color, morphology, and nectar traits. We used RADseq methods to infer phylogenetic relationships in a sample of Penstemon species and found ~12 transitions from bee to hummingbird adaptation but no clear cases of reversal. To explore the genetic architecture of a shift from bee- to hummingbird-adaptation, we performed QTL mapping in a cross between the hummingbird-adapted P. barbatus and its bee-adapted sister species P. neomexicanus. We found evidence that single loci of large effect underlie transitions in flower color and nectar volume, two traits that are critical in initially attracting hummingbirds, suggesting mutations of large effect may spur an initial shift to hummingbird pollination. We also found individual loci may pleiotropically influence multiple floral morphological traits, which may accelerate evolutionary shifts to hummingbird pollination. We identified the genetic basis for red flowers in P. barbatus to be loss-of-function to an anthocyanin pathway gene. We examined this gene in 12 additional hummingbird-adapted Penstemon species and found that parallel shifts to red flowers consistently involve degenerative mutations to this gene – such mutations may have a large mutational target size, but be difficult to reverse. Together, our data suggest that the evolution of hummingbird adaptation in Penstemon may be genetically 'easy', but possibly are difficult to reverse.

8.45-9.00 Evangeline Ballerini (University of California, Santa Barbara)
Identifying the genetic basis of morphological variation in columbine (*Aquilegia*) flowers
Columbines (genus Aquilegia) represent a textbook example of adaptive radiation. Evidence suggests that the evolution of floral nectar spurs in the genus acted as a key innovation promoting pollinator interactions. These pollinator interactions can in turn stimulate floral morphological diversity that is thought to play a critical role in the process of speciation, as many of the floral traits that vary between taxa strongly influence pollination biology and promote assortative mating. We employ a QTL mapping strategy using low coverage whole

genome sequencing to genotype plants at hundreds of thousands of loci across the genome in order to identify the genomic regions controlling variation in species specific traits such as floral color and nectar spur length between A. formosa and A. pubescens, two closely related species with different pollination syndromes (hummingbird and hawkmoth, respectively). In order to understand the genetic basis of spur development, we also use this strategy to map the locus responsible for nectar spur loss in the sole spurless columbine species, A. ecalcarata. This QTL mapping approach, combined with additional data from transcriptome sequencing and population resequencing studies, has allowed for the identification of focal loci critical to the process of speciation in the genus Aquilegia.

9.00-9.15 Alma Pineyro-Nelson (University of California, Berkeley)

Evolution of the F-box gene *UNUSUAL FLORAL ORGANS* in petaloid monocots: Implications for differential petal and stamen development

Within flowering plants, the floral organs that display the greatest array of lineage-specific morphological variations are the petal and stamens. These organs can be subject to differential organ growth, expansion or abortion; variable adnation patterns, stamen laminarization, as well as in extreme cases, inversion on the position of the stamens with respect to the carpels (Lacandonia). Such modifications have likely played a key role in the adaptive radiation of angiosperms, as variations in these organs have been correlated with shifts in pollination syndromes and reproductive systems. The molecular evolution and comparative expression patterns of DEFICIENS (DEF) and GLOBOSA (GLO) -two MADS-box type II transcription factors necessary for petal and stamen development- have been extensively studied across flowering plants. Variations in their spatio-temporal patterns of expression have been correlated with modifications in the floral ground plan in different angiosperm lineages, but one of the key regulators of the early expression of DEF, the F-box protein Unusual Floral Organs (UFO), has been largely understudied. This is particularly so in “petaloid” monocots, that exhibit a wide range of morphological variants with respect to other angiosperms in both petal and stamen development. In this work we present an analysis of the molecular evolution of UFO-like sequences from a large array of monocot taxa and discuss their implications in the underlying Gene Regulatory Networks controlling floral organ development.

9.15-9.30 Cecilia Zumajo Cardona (University of Antioquia, Medellin, Colombia)

Evolution of the *APETALA2* gene lineage in seed plants

AP2/ERF genes are exclusive to vascular plants, classified into the AP2-like and ERF-like clades. The AP2-like includes two

gene groups: the ANT and the euAP2 genes with a miR172 binding site. Arabidopsis has two paralogs in the euAP2 clade, namely APETALA2 (AP2) and TARGET OF EAT3 (TOE3) that control flowering time, meristem determinacy, sepal and petal identity and fruit development. Functional characterizations are sparse outside Brassicaceae, however, euAP2 genes seem to be functionally divergent, as they control fruit development in tomato, but regulate inflorescence meristematic activity in maize. We studied the evolution and expression patterns of euAP2 genes to assess large scale and local duplications and evaluate protein motifs likely related with functional changes across seed plants. We sampled euAP2 genes from seed plants and have found a single major duplication resulting in the AP2 and TOE3 clades in the Brassicaceae and a few taxon-specific duplications. Some euAP2 genes have lost the miR172 binding site, but most have eight protein motifs from which two were previously identified and six are new and reported for the first time. Proteins predating the duplication are more similar to AP2 than TOE3. Expression data shows a shift from restricted expression in leaves, carpels and fruits in early diverging angiosperms and Asterids to a broader expression of euAP2 genes in leaves, all floral organs and fruits in Rosids. Altogether, our data shows a functional trend where the canonical A-function (sepal and petal identity) is exclusive of Brassicaceae and is likely not maintained in non-model angiosperms.

9.30-9.45 Marianna Benitez (Universidad Nacional Autonoma de Mexico UNAM)

Physicochemical factors in the organization of multicellular aggregates and plants

Since the formulation of the Modern Synthesis, the causes of phenotypic variation and innovation, as sources of evolution have been mainly associated to changes in the DNA sequence. However, diverse avenues of theoretical and empirical research are showing that our view of the possible causes and processes by which phenotypic diversity originates needs to be expanded. Indeed, it has been acknowledged that ecological, social, developmental, as well as generic physicochemical processes, may contribute to phenotypic variation and innovation, just as the genome and changes therein do. One of the major evolutionary innovations regarding phenotypes is the appearance of multicellularity in different lineages. Multicellular organisms are not only the aggregation or incomplete separation of cells, but also involve some sort of differentiation, metabolic integration, and the appearance of new systemic properties and levels of selection. The generation of these features is now being studied with a diversity of causes in consideration. We focus on the potential role of physicochemical aspects on the formation and patterning of multicellular arrangements in lineages that, while

evolutionary very distant, might exhibit suggestive commonalities. We illustrate these ideas in a couple of microbial and plant model systems -- the formation of fruiting bodies in Myxococcus xanthus and cell-fate determination in the leaves of Arabidopsis thaliana, respectively -- and discuss how mechanical and chemical coupling of cells and the combination of the so-called Dynamical Patterning Modules (DPMs) may lead to the formation of recurrent patterns in aggregates of unicellular entities and in plants.

9.45-10.00 Deirdre Lyons (Duke University)

Evolution of the blastopore: Fate mapping, live-imaging, and gene expression analyses during gastrulation in the snail *Crepidula fornicata*

Gastrulation is critical for metazoan development, directly linked to germ-layer segregation, axis establishment, and gut formation. For example, the fate of the blastopore, where gastrulation takes place, varies among species: it can become the mouth (protostomy), the anus (deuterostomy), both mouth and anus (amphistomy), or neither. Such variation suggests that changes in gastrulation influenced body-plan evolution, but the mechanism is debated, partly because it is difficult to homologize the blastopore in distantly related taxa. Spiralians offer a means to address this question, because although the fate of the blastopore varies between species, they share a stereotyped early cleavage pattern. This makes it possible to identify unambiguously homologous cell lineages, allowing direct comparison of cells around the blastopore. No modern study has followed the contribution of cells surrounding the blastopore to the mouth and anus in spiralians. Our study in the snail Crepidula is the first to do so. Using cell-labeling and live-imaging, we observed that anterior blastopore lip cells undergo a unique epithelial-to-mesenchymal transition, before making a novel spiralian germ layer, the ectomesoderm. The posterior blastopore lip zippers closed as cells undergo a form of convergence and extension; in the process, cells that later make the anus are displaced from the blastopore. Anterior, posterior, and lateral blastopore lip cells make the mouth. Combining these lineage studies with gene expression analyses, we constructed a fate map linking regulatory factors to specific cell lineages. These data elucidate the evolution of blastopore morphogenesis in spiralians, and by extension, in metazoans in general.

10.00-10.30 COFFEE BREAK

10.30-12.00 Concurrent Talks Session Bi

(*Chair: Ann Burke, Wesleyan University*)

Krutch Theater

10.30-10.45 Sylvain Marcellini (University of Concepcion)

Ancient expression patterns, new enhancers: A comparative study of shark and frog collagen genes provides insights regarding the origin and evolution of the skeletal regulatory network

BACKGROUND: Members of the fibrillar collagen family are major components of the cartilage and bone matrix and are intimately linked to skeletal evolution. Nevertheless, deciphering the evolutionary history of this family still requires a deeper understanding of fibrillar collagen expression patterns and transcriptional enhancers in a variety of vertebrate groups. To tackle this issue, we have adopted a double approach and 1) compared the skeletal expression of shark and amphibian col1 and col2 orthologues and 2) explored the regulatory landscape of amphibian osteoblasts. METHODS: We performed RNA-seq, RT-qPCR and in situ hybridization on developing skeletal elements of the shark Scyliorhinus canicula and the frog Xenopus tropicalis. Enhancers were predicted by detecting four histone marks by ChIP-seq performed on freshly extracted, uncultured, frog osteoblasts. Putative collagen enhancers were functionally validated in transfected primary osteoblasts and transgenic larvae. RESULTS: A robust expression of col1 is observed in the perichondrium of sharks and amphibians both before and after mineralization. Remarkably, vertebral columns from both species display an overlooked, col2-negative and heavily mineralized type of cartilage. We functionally validate an active Xenopus osteoblastic enhancer invaded by amphibian-specific repeats and located 40kb upstream of col1. CONCULSIONS: A col2-negative hypercalcified cartilage and a col1-positive mineralized bone/perichondrium emerged before the chondrichthyan-osteichthyan split. The presence of highly repeated amphibian-specific sequences within the col1 upstream enhancer suggests that it evolved recently. We therefore propose that transcriptional output can be maintain for long period of time in spite of a dramatic turnover of the skeletal regulatory landscape. FUNDING: FONDECYT 1151196

10.45-11.00 Jessica Gray (Harvard Medical School)

The evolving roles of miRNA regulation in development

The class of short non-coding RNAs known as miRNAs are intriguing candidates for the study of developmental regulatory changes through evolution. Since an individual miRNA can potentially target a large number of genes, miRNAs are capable of broad manipulation of signaling pathways or development processes. Indeed, miRNAs are well established as key regulators of development in model organisms. However, while patterns of sequence conservation and tissue expression of miRNAs across bilateria have been linked to morphological complexity, the functional roles of these miRNAs

in the development of a wide range of organisms remain unknown. The question remains whether changes in miRNA targets and functions play a role in evolution or instead are uniquely regulated in different lineages. We are establishing the direct-developing hemichordate Saccoglossus kowalevskii as a model for addressing this question in the deuterostome lineage. Towards that end we have profiled miRNA expression across development and generated genome-wide target predictions. We have also established methods for in vivo functional perturbations in order to identify the developmental signaling pathways that these miRNAs regulate. With these tools we are able to combine expression, phenotype and target information to generate testable hypotheses regarding Saccoglossus miRNA regulatory networks. The targets and functions of these developmental miRNAs are being compared with known homologs and functional counterparts, with ongoing investigations focusing on neural miRNAs such as miR-124. Our goal is to understand miRNA function in hemichordates to provide insight into how miRNA regulation in development has changed through evolution.

11.00-11.15 Brent Hawkins (Harvard University)

A single mutation reveals latent capacity for limb-like development in the zebrafish

The diversification and specialization of the paired appendages are hallmarks of vertebrate evolution. In the lineage leading to tetrapods, the appendicular skeleton was elaborated along the proximal-distal (PD) axis by adding articulated skeletal elements to form the stylopod (humerus), zeugopod (radius/ulna), and autopod (wrist/hands) of the limbs. This tripartite skeletal pattern has remained constant during the 360 million years of tetrapod evolution. In contrast, the teleost fish lineage shows a reduced appendicular skeletal pattern with a diminutive endochondral skeleton, consisting of only proximal radials and small, nodular distal radials along the PD axis. This pattern is canalized and has persisted over 250 million years of teleost evolution. Using a forward genetic approach in the zebrafish, we have discovered an adult-viable, dominant mutation that results in the acquisition of supernumerary radial bones located between the proximal and distal radials. Unlike the proximal radials, these extra elements have both proximal and distal growth zones and articulate with proximal and distal radials. Ontogenetic analyses reveal that the new elements develop from the branching and splitting of cartilaginous condensations in a fashion similar to that seen in tetrapod limb development. Unexpectedly, the extra elements are sometimes directly connected to musculature, which is not observed in wild type radials. An analysis of early development shows modification of known limb developmental gene networks in mutant fins. The genetic alteration in this mutant reveals the latent capacity for skeletal elaboration in fins of fishes and may

inform our understanding of 'limbness' and the fin to limb transition.

11.15-11.30 Yi-Hsien Su (Academia Sinica)

Conserved and novel BMP/Admp circuits control dorsoventral polarity in ambulacrarians

The spatially opposed expression of Antidorsalising morphogenetic protein (Admp) and BMP signals controls dorsoventral (DV) polarity in both chordates and planarians, and this regulatory circuit has been considered an ancient mechanism in Bilateria. Here, we show that in addition to the conserved admp gene that constitutes the ancient circuit, a second admp gene (admp2) is present in Ambulacraria (Echinodermata+Hemichordata). We show that the two admp genes are under opposite transcriptional control by BMP signals in both sea urchin and acorn worm embryos and thus are expressed on the opposite sides along the DV axis. We further demonstrate that both Admp proteins reinforce BMP signalling in sea urchin and zebrafish embryos for DV patterning. Our results suggest that both conserved and novel BMP/Admp regulatory circuits control the DV polarity in ambulacrarian embryos. We also show that this novel admp2 gene is not found in other bilaterian genomes that we have searched, except in two species of marine worms belonging to Xenoturbellida and Acoelomorpha, which have been proposed to form a clade called Xenacoelomorpha with uncertain phylogenetic position either at the base of Bilateria or within Deuterostomia as a sister group to Ambulacraria. The observed phylogenetic distribution of admp genes implies a duplication event in the common ancestor of Bilateria, following which both protostome and chordate lineages have lost the admp2 genes.

11.30-11.45 Arnaud Martin (University of California, Berkeley)

***WntA* is a morphogene essential for butterfly wing pattern formation and diversification**

Most butterfly wing patterns form "symmetry systems", visible as successive color stripes mirrored around organizing centers that run between the anterior and posterior wing margins. It has been long thought that the diffusion of signaling molecules with concentration-dependent outputs, or "morphogens" could explain the emergence of discrete boundaries during pattern formation. I will present evidence that the Wnt-family gene *WntA* has the properties of such predicted morphogens. *WntA* was originally mapped as a locus driving adaptive phenotypic variation in the shape of the symmetry systems of several butterflies. In all butterfly species that were tested, *WntA* expression is tightly associated during larval wing disk development with discrete expression domains that map presumptive patterns. Heparin injections, predicted to enhance Wnt-signaling, result in specific expansions of Wnt-positive

patterns. More importantly, somatic of depletion of WntA using CRISPR-Cas9 mutagenesis generated butterflies lacking *WntA*-positive symmetry systems, supporting a morphogen model. Our data establish direct evidence between morphogens, pattern formation, and the subsequent diversification of forms in nature.

11.45-12.00 Paul Gonzalez (Stanford University)

Comparing axial patterning across divergent life histories: Data from the indirect-developing hemichordate Schizocardium

How do patterning mechanisms evolve when life cycles become more or less complex? Most marine invertebrates have a biphasic life cycle where the embryo first develops into a planktotrophic larva (indirect developers), while others develop directly into a small-scale version of the adult (direct developers). Because larval and adult body plans are often radically different, these differences in life cycles may be paralleled by differences in patterning mechanisms. Comparing closely related species with morphologically similar adult stages but contrasting life history strategies may allow us to determine whether axial patterning mechanisms differ between direct and indirect developers, independent of their adult body plan. Here we describe axial patterning mechanisms in the indirect-developing hemichordate Schizocardium californicum. We describe the expression patterns of transcription factors with known function in anteroposterior (AP) patterning in other deuterostomes, as well as preliminary data on the function of some of the main signaling pathways that establish AP and dorso-ventral polarity. We show that these mechanisms are more similar to the direct-developing hemichordate Saccoglossus, which has a similar adult body plan but an abbreviated life cycle, than to echinoderms, which have highly derived adult body plans but similar larvae. These results suggest that evolutionary transitions between indirect and direct development do not necessarily require major changes in early patterning of the embryo.

10.30-12.00 Concurrent Talks Session Bii

(*Chair: Trisha Wittkopp, University of Michigan*)

Garden Room

10.30-10.45 Erin Jarvis Alberstat (University of California, Berkeley)

Genetic interactions between the Hox genes *Scr, Antp*, *Ubx*, *abd-A*, and *Abd-B* in the amphipod crustacean *Parhyale hawaiensis*

Hox genes are crucial in establishing segment specialization, including limb identity, along the anterior-posterior. They mediate segmental identity by regulating the expression of

downstream targets, as well as fine-tuning the expression or function of each other through various levels of cross-regulation. Here we investigate regulatory interactions among the five Hox genes expressed within the thorax and abdomen of the amphipod crustacean *Parhyale hawaiensis—Sex combs reduced (Scr), Antennapedia (Antp), Ultrabithorax (Ubx), abdominal A (abd-A)*, and *Abdominal B (Abd-B)*. We use RNAi and CRISPR/Cas9 to respectively knockdown or knockout the expression of these genes, and visualize concurrent shifts in the expression patterns of neighboring Hox genes using antibody staining and *in-situ* hybridization, and test our interaction models through the generation of double mutants. Our results show that changes to Hox domains cause corresponding shifts in neighboring Hox boundaries (for example, *Abd-B* knockout causes a posterior shift in the expression domains of *Ubx* and *Antp),* but no shift in the boundary of *abdA*. This suggests that reverse facing walking legs are specified by the combination of *Ubx*, *Antp*, and *abdA*, while forward walking legs are specified by the combination of *Ubx* and *Antp*. This model was then tested by creating double mutants of *abdA*+*AbdB* and *Ubx*+*abdA*. Thus, the integration of new combinations of Hox expression may be an important mechanism for generating unique segmental identities and appendage diversity in malacostracan crustaceans.

10.45-11.00 Emily Delaney (University of California, Davis)

Regulation of a female-limited color dimorphism in *Drosophila serrate*

In spite of their largely shared genome, males and females can differ dramatically in form and behavior. Though sexually dimorphic traits have been catalogued in a diversity of plant and animal taxa, our understanding of the genetic mechanisms that lead to these alternative states has lagged. To better comprehend how sex-specific gene regulation arises and contributes to dimorphism, we are studying a female-limited pigmentation dimorphism in *Drosophila serrata*. In this species, females are either lightly or darkly pigmented on their abdomen, whereas males are always lightly pigmented. We found a single Mendelian locus with a dominant dark allele controls abdominal pigmentation. Interestingly, males with dark genotypes do not manifest dark pigmentation. We mapped this locus to a ~700 kb region on the 2nd chromosome containing a cluster of SNPs just upstream of the transcription factor POU domain motif 3 (*pdm3*). We tested *pdm3* function and found that it represses pigmentation. Currently, we are characterizing the molecular differences between light and dark *pdm3* alleles and testing their interactions with the *Drosophila* sex differentiation and pigmentation pathways to determine: (1) the role of *pdm3* in female dimorphism, and (2) the regulatory network that restricts dark pigmentation to females, not males. To complement our molecular analyses, we are also testing

the fitness of each sex and genotype to identify forces that may maintain both *pdm3* alleles in natural populations. Together, our integrative research into the genetic mechanisms controlling abdominal pigmentation in *D. serrata* will elucidate how, and possibly why, sex-limited traits evolve.

11.00-11.15 Yi-Jyun Luo (Okinawa Institute of Science and Technology Graduate University)

The brachiopod genome of *Lingula anatina* provides insight into the evolution of lophotrochozoans and calcium-phosphate-based biomineralization

An abundance of the Silurian Period, Lingula fossils with morphology very similar to that of extant species inspired Darwin with the idea of "living fossils." Although they superficially resemble bivalve molluscs, lingulid brachiopods show unique features, including radial cleavage and dorsoventrally oriented shells. In particular, their shells are composed of calcium phosphate and collagen fibers, characters shared only by evolutionarily distant vertebrates, one of the biggest mysteries of metazoan evolution. To gain insights into brachiopod evolution, we decoded the 425-megabase genome of Lingula anatina. Comprehensive phylogenomic analyses place Lingula close to molluscs, but distant from annelids. Among lophotrochozoans, Lingula shows the slowest evolutionary rate of genes associated with basic metabolism. Its gene number increased to ~34,000 by extensive expansion of gene families, especially those associated with shell formation. In addition, we found that Lingula shared shell formation-related genes and mechanisms similar to molluscs, such as chitin synthase and bone morphogenetic protein (BMP) signaling. Although Lingula and vertebrates share similar hard tissue components, our genomic, transcriptomic, and proteomic analyses showed that Lingula lacks genes involved in bone formation, indicating a classical example of convergent evolution. Furthermore, we showed that Lingula has experienced domain combinations to produce shell matrix collagens with epidermal growth factor (EGF) domains and carries lineage specific shell matrix proteins, such as alanine-rich fibers. We propose that gene family expansion, domain shuffling, and co-option of genes appear to be the genomic background of Lingula's unique biomineralization.

11.15-11.30 Eric Camino (University of Dayton)

The evolutionary origination and diversification of a dimorphic gene regulatory network through parallel innovations in cis and trans

The origination and diversification of morphological characteristics represents a key problem in understanding the evolution of development. Morphological traits result from gene regulatory networks (GRNs) that form an interconnected

landscape of transcription factors, which regulate multiple *cis*-regulatory element (CRE) sequences to control the coordinated expression of differentiation genes. The formation and modification of GRNs must ultimately be understood at the level of individual regulatory linkages (i.e. transcription factor binding sites within CREs) that constitute the network. Here, we investigate how elements within a network originated and diversified to generate a broad range of abdominal pigmentation phenotypes among *Sophophora* fruit flies. Our data indicates that the coordinated expression of two melanin synthesis enzymes, Yellow and Tan, recently evolved through novel CRE activities that respond to the spatial patterning inputs of Hox proteins and the sex-specific input of Bric-à-brac transcription factors. Once established, these newly evolved activities were largely modified by evolutionary changes in the network's *trans*-regulatory landscape to generate large-scale changes in pigment pattern. By elucidating how *yellow* and *tan* are connected to the abdominal *trans*-landscape, we discovered that the *yellow* and *tan* abdominal CREs are composed of distinct regulatory inputs that exhibit contrasting responses to the same Hox proteins and Hox cofactors. These results provide an example in which CRE origination underlies a recently evolved novel trait, and highlights how coordinated expression patterns can evolve in parallel through the generation of unique regulatory linkages.

11.30-11.45 Pamela Windsor-Reid (University of Alberta)

The role of Wnt signaling in the development of polarity and the canal system in sponges

The role of Wnt signaling in body plan polarity is a recurring theme in all metazoans; studying this pathway in one of the earliest arising phyla – sponges – offers insight into early animal body plan evolution. The sponge body plan appears to lack polarity and consists of a series of feeding canals that terminate at the osculum, or vent, of the sponge. Comparison with other animal body plans is possible since the osculum effectively polarizes the sponge body plan, and larvae have antero-posterior polarity with respect to swimming direction. In sponge larvae some *wnt* genes are expressed at the posterior pole, and in adults some *wnt* genes are expressed at the tip of the osculum. Bioinformatic analysis shows that Wnt signaling components are present across sponge taxa, but sponge Wnts fall outside of the known Wnt subfamilies. While bioinformatic and expression studies in sponges are becoming increasingly common, functional studies are lacking. We previously showed that inhibition of GSK3 using either lithium chloride or alsterpaullone caused multiplication of the osculum. By inhibiting several other members of the Wnt pathway – Fz, Dsh, and β-catenin/TCF – we show oscula are absent. Antibodies to β-catenin and Dsh in progress should show readout of Wnt signaling in both embryonic and adult sponges.

Our findings suggest that Wnt signaling controls polarity in sponges by the formation of oscula and a polarized filtration system. Establishment of polarity in sponges by the Wnt pathway suggests it was instrumental for evolution of complexity in the metazoan body plan.

11.45-12.00 Sofia Casasa (Indiana University)

Insulin Signaling's role in mediating nutrition-responsive growth in the polyphenic beetle *Onthophagus Taurus*

The developmental-genetic mechanisms underlying phenotypic plasticity and their contribution to evolution are of major interests to biologists. We are investigating the role of the insulin signaling pathway, known to link nutrition to growth in a wide range of organisms, in the ontogeny and evolution of polyphenic development in the beetle *Onthophagus taurus*. In this species males exhibit a nutrition-sensitive male dimorphism in which high nutrition results in fully horned fighter males, whereas development under low nutrition conditions result in hornless sneaker males. We executed RNA interference mediated gene function analyses to study the role of two cardinal components of the insulin signaling pathway, the insulin receptor (InR, which promotes cell proliferation when activated by insulin-like peptides in the presence of high nutrition), and FOXO (a growth inhibitor downstream of the insulin receptor which is activated during nutrient stress). We show that both manipulations significantly alter the highly sigmoidal body size-horn length allometry typical of this and many other species. Specifically, *InR*-RNAi significantly delays horn formation to larger body sizes, whereas *FOXO*-RNAi both induces horns in low nutrition males and reduces them in high nutrition individuals, thereby linearizing the allometry. This raises the possibility that FOXO may have played a critical role in the developmental evolution of complex allometries from linear scaling-relationships. More generally, our results suggest that the insulin signaling pathway plays a key role in regulating nutrition-dependent growth and horn polyphenism in *Ontophagus taurus* and possibly many other taxa.

12.00-13.30 LUNCH
Dining Center

12.00-13.30 Informal Workshop/Lunch

(*Cassandra Extavour, Harvard University*)

People of color in evo-devo

Join us for the people of color lunch! The goal is to meet informally over lunch to share experiences, discuss any areas of concern, and encourage collegiality and support among self-identified people of color in the Evo-Devo field. The conversation will be mediated by Cassandra Extavour. There is no need to pre-register, just drop by and join us!

13.30-14.30 Plenary Talks Session 4
(*Chair: Elena Kramer, Harvard University*). This plenary session is sponsored by Society of Developmental Biology.

Krutch Theater

13.30-14.00 Tamara Franz-Odendaal (Mount Saint Vincent University)
The Vertebrate Skull: Understanding the evo-devo-eco of the sclerotic ring
The complexity of the vertebrate skull has intrigued both evolutionary biologists and developmental biologists for centuries. We focus on the neural crest derived bones that surround or are associated with the eye, an integral part of the sensory system for most vertebrates. One particularly intriguing skeletal element is the sclerotic ring. This element is situated within the eye of many extant vertebrates and has a long evolutionary history. The ring is composed of a variable number of individual bony plates within the sclera of the eye and is thought to aid in visual accommodation. Through gross morphological and experimental developmental biology studies, we show that while this element is variable in shape and size within different lineages, it is highly constrained in its development. Lineages that have secondarily lost the sclerotic ring include placental mammals, snakes, several squamates and some teleosts. Using a variety of approaches that include analysing vasculature, surgically over expressing inhibitors for major signalling pathways in development (the Hedgehog and Transforming Growth Factor β families), gene expression, and DiI tracking, my research group has gained significant insight into how the sclerotic ring develops, is constrained in development and how variation arises. We have optimised both an ex ovo and in vitro systems for culturing the embryo outside of the shell and for culturing the eye without the rest of the embryo. This multi-faceted approach that includes developmental and evolutionary perspectives has led to major advances in our understanding of this intriguing element.

14.00-14.30 Julia Bowsher (North Dakota State University)
The evolution of a novel sexual ornament: Integrating mechanistic explanations across species
Many novel traits are strongly influenced by sexual selection. Although sexual selection is a powerful evolutionary force, underlying genetic interactions can constrain or facilitate evolutionary outcomes. Male flies in the family Sepsidae have novel abdominal appendages that have a complex pattern of gain and loss across the clade. This gain and loss of abdominal appendages is correlated with changes in cell proliferation in abdominal imaginal cells, which give rise to these abdominal appendages. In the sepsid Themira biloba, transcriptomic analysis has identified genes that are up-regulated in these cells, including those involved in bristle formation. Experimental manipulation of the bristles on the

abdominal appendages indicates that long bristles are necessary for successful mating. Measurement of trait variance indicates bristle length is highly correlated with other appendage traits. Taken together, these results suggest that sexual selection on bristle length has facilitated the evolution of abdominal appendages through genetically correlated changes in other traits such as bristle number and abdominal appendage length.

14.30-15.30 Poster Session (Posters 1-179; see pages 50-143 of this booklet) & BREAK

Ginkgo Courtyard

15.30-18.00 Plenary Talks Session 5

(*Chair: Leslie Pick, University of Maryland*). This plenary session is sponsored by JEZB: Molecular Developmental Evolution.

Krutch Theater

15.30-16.00 Rachel Collin (Smithsonian Tropical Research Institute)

Convergent Evolution of Alternate Developmental Phenotypes in Calyptraeid Gastropods

Nutritive embryos, which arrest their development and serve as nutrition for their developing siblings, are found in a range of animal groups including ants, frogs and sharks. Among marine invertebrates they have evolved frequently in both gastropods and polychaetes. In particular nutritive embryos in calyptraeid gastropods provide an ideal system for the integrative study of the ecology, evolution and development of this polyphenism. Diverse lines of study suggest that nutritive eggs may function to produce particularly large offspring, and/or to increase variance in offspring size. However the ecological factors that have lead to the evolution of nutritive embryos remain obscure. Despite this, phylogenetic study provides evidence that nutritive embryos have evolved at least 10 times in calyptraeids. Observations of nutritive embryos and the developing embryos that consume them bring to light a number of strong evolutionary parallelisms and convergences in the overall morphology of both kinds of embryos among most calyptraeids with nutritive embryos. Unique types of embryos have also evolved. For example *Crepipatella dilatata* has oophagy where the nutritive embryos do not develop, and *C. philippiana* and *M. monoxyla*, two distantly related taxa, have both evolved a system with only a single highly modified embryo per capsule. In depth genomics and evo-devo study of one species, *Crepidula navicella*, show distinct gene expression patterns in nutritive and viable embryos, highlighting the specialized developmental pathway that leads to the production of nutritive embryo phenotypes.

16.00-16.30 Frietson Galis (Naturalis Biodiversity Center)

Constraints on the evolvability of the vertebral column in mammals

The spectacular diversification of the vertebrate body plan since the Ordovician is to an important extent due to the evolvability of the segmented vertebral column. Alongside the remarkable evolutionary diversification, there has also been impressive conservation of the vertebral column. The number of vertebrae within specific vertebral regions is for instance highly conserved in many vertebrate taxa. Earlier, we have shown that changes of cervical and trunk numbers commonly occur in humans and other mammals, but are strongly selected against in many, but not all, taxa. The selection is due to unavoidable pleiotropic effects. We present new data on extant and extinct species and show how the strength of the selection results from an interaction between developmental and biomechanical constraints. Furthermore, we will discuss our results in the light of robustness and canalization.

16.30-17.00 BREAK

16.30-17.00 Informal Workshop/Break

(*Angelika Stollewerk, Queen Mary University of London*)

Women in Science

Join us for the women in science coffee break! The aim of this event is to encourage networking among female scientists at all levels of their career. This is a great opportunity to discuss strategies for developing your career and join forces to overcome the difficulties women face in academia. The event will be coordinated by Angelika Stollewerk who is an active member of the UK gender equality network (Athena Swan) and chair of the Athena Swan committee at her department at Queen Mary University of London.

17.00-17.30 James Hanken (Harvard University)

Evolutionary innovation and conservation in the embryonic derivation of the vertebrate skull

Development of the vertebrate skull has been studied intensively for more than 150 years, yet many essential features remain unresolved. One such feature is the extent to which embryonic derivation of individual bones is evolutionarily conserved or labile. Such data have been particularly difficult to obtain for amphibians, and especially frogs, in which bones don't differentiate until metamorphosis, which may not occur until weeks, months, or even years after hatching. We performed long-term fate mapping using GFP-transgenic strains of both axolotl (Ambystoma mexicanum) and clawed frog (Xenopus laevis) to document the contribution of embryonic neural crest to the adult osteocranium in each species. The pattern of derivation from individual migratory

crest streams in the axolotl is strikingly similar to that in amniotes; it likely represents the ancestral condition for tetrapods, which is retained in most extant clades. Unexpectedly, the pattern in Xenopus is much different. It may constitute a unique condition that evolved after anurans diverged from other amphibians. Such changes may reveal an unappreciated relation between life history evolution and cranial development and exemplify "developmental system drift," in which interspecific divergence in developmental processes that underlie homologous characters occurs with little or no concomitant change in adult phenotype. They also caution against the use of ontogenetic data as an exclusive or infallible criterion for evaluating homology, which may obscure, rather than reveal, important trends in comparative and evolutionary biology.

17.30-18.00 Angela Hay (Max Planck Institute for Plant Breeding Research)
Morphomechanical innovation drives explosive seed dispersal
How mechanical and biological processes are integrated across different scales to create complex traits is largely unknown. In this work, we combine biological, mathematical, and computational approaches to understand the mechanical basis for explosive seed dispersal - a key life history trait underpinning invasive behavior in the common weed *Cardamine hirsuta*. We have exploited the experimental tractability of *C. hirsuta* - a close relative of the model organism *Arabidopsis thaliana* – to understand the mechanism of explosive pod shatter and provide insights into the origin of this striking trait.

18.00-19.30 DINNER
Dining Center

19.30-20.30 Plenary Talks Session 6
(*Chair: Pam Diggle, University of Connecticut*). This plenary session is sponsored by Society of Developmental Biology.
Krutch Theater

19.30-20.00 Igor Schneider (Universidade Federal do Para)
Fin regeneration in the South American lungfish: Insights into the evolution of vertebrate limb regeneration
Approximately 400 million years ago, lobe-finned fish colonized land, giving rise to all four-legged vertebrates, known as tetrapods. Of all anatomical features necessary for life on land, the origin of limbs with digits is perhaps the most strongly associated with this evolutionary transition. Among tetrapods, salamanders are unique in their capacity to regenerate a missing limb throughout their adult life. As a result, urodele amphibians have become the experimental model organism of choice for studies on limb regeneration. Over the past couple

of decades, research efforts have focused on revealing how limb regeneration occurs, yet another important question has gained attention in recent years: why is limb regeneration limited to salamanders? Appendage regeneration may be an ancestral trait of tetrapods, or it may have independently evolved in urodele amphibians. To address this, we have focused on studying fin regeneration in the South American lungfish, Lepidosiren paradoxa. Lungfishes are the sister group of tetrapods and are capable of regenerating their fins. Our histological analysis reveals that, as seen in salamanders, lungfish regeneration involves the formation of a proliferative blastema that remains in contact with epidermal cells due to the lack of a basement membrane. Our transcriptome analysis of regenerating lungfish fins shows the up-regulation of developmental genes and down regulation of muscle genes, a trend also observed in regenerating limbs of axolotls and froglets. Combined with analysis of cell proliferation and shh signaling inhibition, our data is consistent with the hypothesis of an ancient origin of a limb regeneration genetic program.

20.00-20.30 Kim Cooper (University of California, San Diego)

Multiple phylogenetically distinct events shaped the evolution of limb skeletal morphologies associated with bipedalism in the jerboas

Recent rapid advances in experimental biology have expanded the opportunity for interdisciplinary investigations of the evolution of form and function in non-traditional model species. We take advantage of the extraordinary morphological diversity of the rodent superfamily Dipodoidea, including the bipedal jerboas, to experimentally study the developmental mechanisms and biomechanical performance of a remarkably divergent limb structure. Here, we place multiple limb character states in a biomechanical and phylogenetic context. We then identify temporal patterns of evolution, approximate genetic complexity, and build a framework for identifying the developmental mechanisms of limb evolution. While obligate bipedalism arose once in the ancestor of extant jerboas, we found that digit loss, metatarsal fusion, forelimb-hindlimb proportions, and within hindlimb proportions all evolved independently of one another. Digit loss occurred through at least two distinct developmental mechanisms, and elongation of the hindlimb relative to the forelimb is not simply due to growth mechanisms that change proportions within the hindlimb. Furthermore, we found strong evidence for punctuated evolution of allometric scaling of hindlimb elements during the radiation of Dipodoidea. Our work demonstrates the value of leveraging the evolutionary history of a clade to establish criteria for identifying the developmental genetic mechanisms of morphological diversification.

20.30-22.00 Future of Evo-Devo Panel Discussion

(*Co-Moderators: Trisha Wittkopp,University of Michigan & Greg Wray, Duke University*)

Please join us for a sure-to-be exciting discussion on the future of our field! The panel is composed of 6 junior and mid-career faculty from around the world and will be moderated by Trisha Wittkopp and Greg Wray. The aim is to encourage maximum audience participation. Our moderators will be asking questions to both the panel and audience like “what do you think are the most exciting discoveries and opportunities in evodevo research?” and “what are the emerging themes, trends, questions, can we be looking forward to?”

Panel members:
Cassandra Extavour (Harvard University, USA)
Johannes Jaeger (KLI Institute, Austria)
Lena Hileman (University of Kansas, USA)
Federico Brown (Universidade de Sao Paulo, Brazil)
Mansi Srivastava (Harvard University, USA)
Abderrahman Khila (Institute of Functional Genomics, France)

Saturday August 8th

7.00-8.30 BREAKFAST
Dining Center

08.30-10.00 Plenary Talks Session 7
(*Chair: Chris Lowe, Stanford University*)
Krutch Theater

8.30-9.00 Robb Krumlauf (Stowers Institute)
Coupling Hox genes to head development in chordate evolution: A story in segments
The hindbrain of jawed vertebrates is a specialized and highly conserved region of the nervous system that serves as a complex coordination center for motor activity, breathing rhythms and many unconscious functions. It also plays a key role in regulating head formation via the generation of neural crest cells. Regional diversity in the hindbrain is achieved through a process of segmentation whereby it is subdivided into repetitive segments called rhombomeres (r) and the Hox family of transcription factors is functionally coupled to this process. A defining feature of the jawed vertebrate phylotype is a highly conserved and well characterized gene regulatory network that governs head patterning by integrating hindbrain segmentation with the generation of segmentally-restricted domains of Hox gene expression to specify the unique identities of rhombomeres and facial structures. Non-vertebrate chordates display nested domains of axial Hox gene expression but lack hindbrain segmentation and the key cis-regulatory elements from jawed vertebrate Hox clusters are not conserved in amphioxus or ascidians. By taking advantage of the unique evolutionary position of the lamprey at the base of vertebrates and performing cross-species regulatory analysis we have uncovered a deep level of conservation in the hindbrain gene regulatory network in lamprey. These data indicate that coupling of Hox gene expression to segmentation of the hindbrain is an ancient trait with origin at the base of vertebrates.

9.00-9.30 James Umen (Donald Danforth Plant Science Center)
From Minus to Males: Coevolution of Sexes and Multicellularity in Volvocine Algae
Distinct male and female sexes have evolved repeatedly in eukaryotes, but the origins of dimorphic sexes and their relationship to mating types in unicellular species are not understood. Volvocine algae are a closely related monophyletic group that includes isogamous species such as *Chlamydomonas reinhardtii*, and oogamous multicellular species such as *Volvox carteri* (Volvox) with sperm-producing males and egg-producing females. Sexual differentiation and mating in volvocine algae are controlled by a multigenic mating

locus (*MT*) with structurally divergent haplotypes. We found that a single conserved transcription factor gene—*MID,* with orthologs present in only the *minus* or male haplotype of each species—evolved from its ancestral role in Chlamydomonas as a mating-type specifier to become a determinant of sperm and egg formation in the Volvox lineage. Transgenic female Volvox with ectopically expressed male *MID* have a pseudo-male phenotype forming functional sperm packets instead of eggs, while transgenic male Volvox with RNAi-knockdowns of *MID* have a pseudo-female phenotype forming functional eggs instead of sperm packets, or differentiating as self-fertile hermaphrodites. The uncoupling of sex chromosome identity from sexual differentiation in pseudo-males and pseudo-females of Volvox reveals antagonistic interactions between the MID pathway and other genes in its male and female *MT* haplotypes that impact sexual development and reproductive fitness. Ongoing work is aimed at functional dissection of MID protein evolution in the volvocine lineage, in the elucidation of MID gene regulatory networks from Chlamydomonas and Volvox, and the identification of male and female fitness genes residing in Volvox *MT*.

9.30-10.00 Manu Prakash (Stanford University)

Life in Flatland: Complex morphogenetic and behavioral traits of a simple basal metazoan

My lab studies Trichoplax adhaerens (Phylum Placozoa) as a model system for physical biology of primitive organisms. It's flat and simple body plan allows experimental manipulation while broad range of size provides a natural cell-number variation. By developing large-field of view live imaging techniques and experimental behavioral assay, we assess where complexity in this simple organism arises both in locomotory behavior and morphogenetic traits as a function of organism size. Using multi-scale techniques from soft-matter physics, here we present a paradigm where simple tissue can paradoxically behave both as a solid sheet but still flow like a two-dimensional fluid.

10.00-10.30 COFFEE BREAK

10.00-10.30 Informal Workshop/Coffee Break

(*Leslie Pick, University of Maryland*)

Participation of persons with disabilities in evo-devo

Join us for a discussion on enhancing the participation of persons with disabilities in science in general and in the Evo-Devo field in particular. The aim of this event is to encourage networking among persons with disabilities at all levels of their career. This is a great opportunity to discuss strategies for developing your career and to join forces to overcome difficulties faced in different countries, regions, institutions and laboratory settings. The event will be coordinated by Leslie

Pick who is a Program Director of an NIH T32 Training Grant in Cell & Molecular Biology and chair of the Department of Entomology at the University of Maryland.

10.30-12.00 Plenary Talks Session 8
(*Chair: Chelsea Specht, University of California, Berkeley*)

Krutch Theater

10.30-11.00 Deniz Erezylimaz (Stony Brook University)

The Dating Game: Species-specific mate preference in Drosophila

Although great advances have been made in identifying developmental changes that give rise to morphological differences between genera, far less is known of how changes in neural development may produce meaningful differences in behavior. As an initial step towards understanding the genetic basis of behavioral evolution, we have applied high-resolution genetic mapping to identify loci that influence Drosophila mating preference. Mate discrimination at courtship in Drosophila is believed to occur through 1) female preference for species-specific courtship songs, and 2) male preference for species-specific female contact pheromones. D. sechellia, and D. simulans, diverged ~242,000 years ago. Crosses between the two species produce viable males and fertile females. While D. sechellia males and D. simulans females mate readily, the reciprocal cross, between D. sechellia females and D. simulans males, is rarely successful. We tested ~1500 D. simulans-D. sechellia recombinant females in 2-choice mating assays and used a high-resolution seq-based method to identify regions of the genome that determine species-specific mate preference in females. Our analysis implicates two enzymes that produce the pheromone, 7,11 heptacosadiene (7,11-HD) in D. sechellia females, but not D. simulans females. These data suggest that for this species pair, male preference for female pheromones, but not female preference for male courtship songs, determines whether copulation will occur. We are currently using genome editing, and tissue specific expression to test this prediction. Finally, we are using the same approach to identify genes that underlie differences in male preference for species-specific pheromones in females.

11.00-11.30 Alexa Bely (University of Maryland)

Evolution of regeneration in annelids: Testing for physiological correlates

The ability to regenerate body parts is highly variable across animals suggesting this ability has been evolutionarily highly labile. However, little is known about where across the animal tree gains and losses of regeneration have occurred, or what factors might drive the evolution of regenerative ability. We are using ancestral trait reconstructions to infer the pattern of

regeneration evolution across several groups of animals and have identified both likely gains and losses of regeneration ability. Focusing on a group of annelids, we are investigating several possible physiological correlates of regeneration, including starvation resistance, metabolic rate and metabolic stores. Determining where evolutionary changes in regenerative ability have occurred and identifying ecological, morphological, physiological, and molecular correlates of regeneration should shed needed light on the ultimate and proximate processes leading to changes in regenerative ability.

11.30-12.00 Bob Reed (Cornell University)

Evolution in Technicolor: Developmental mechanisms of butterfly wing pattern evolution

Butterfly wing patterns present a useful system to study the origin and evolution of morphological traits. We can trace the histories of specific color pattern elements using extensive phylogenetic knowledge of the butterfly lineage, and we can use powerful new molecular approaches to characterize the genes and mechanisms that underlie changes within and between species. Several general trends are emerging from wing pattern work, including the repeated roles of a handful of patterning genes in both convergent and divergent evolution of complex patterns, the developmental and genetic modularity of color patterns elements, the role of morphogens in translating simple pre-patterns into more complex derived patterns, and a recurring theme of cis-regulatory variation driving pattern evolution. Here I describe our ongoing work to better understand the developmental genetic basis of color pattern evolution in butterflies, with an emphasis on our current efforts to characterize the regulatory elements and networks underlying wing pattern evolution, and our work to understand the evolution of seasonal wing pattern plasticity.

12.00-13.30 LUNCH
Dining Center

12.00-13.30 Informal Workshop/Lunch

(*Cassandra Extavour, Harvard University*)

Queer and LGBT folks in evo-devo

Join us for queer/LGBT lunch. The goal is to meet informally over lunch to share experiences, discuss any areas of concern, and encourage collegiality and support among self-identified LGBT/queer people in the Evo-Devo field. The conversation will be mediated by Cassandra Extavour. There is no need to pre-register, just drop by and join us!

13.30-14.30 Plenary Talks Session 9

(*Chair: Nipam Patel, University of California, Berkeley*). This plenary session is sponsored by JEZB: Molecular Developmental Evolution.

Krutch Theater

13.30-14.00 Matt Rockman (New York University)

How to lose your larva: The transition from planktotrophy to lecithotrophy in the polychaete *Streblospio benedicti*

Developmental processes are phenotypes exposed to natural selection, independent of selection on adult forms. We study a species of polychaete in which selection has shaped two alternative developmental modes. In the planktotrophic morph of *Streblospio benedicti*, females produce large numbers of small eggs that develop into typical planktonic trochophores that feed in the plankton for weeks before settlement. In the lecithotrophic morph, females make small numbers of large eggs that develop into settlement-competent larvae without feeding. The difference between morphs mimics the ubiquitous macroevolutionary transition between indirect and direct development in marine invertebrates. Using classical and molecular quantitative genetics and population genetics, we are learning the genetic basis for developmental variation in this species.

14.00-14.30 Stacey Smith (University of Colorado Boulder)

Mechanisms of flower color convergence across multiple evolutionary time scales

Genetic and developmental studies of phenotypic evolution have uncovered many cases in which the same genes and, in some cases, even the same mutations, underlie the independent evolution of similar phenotypes in distantly related lineages. One explanation for such genetic convergence is selective filtering of mutations, whereby a certain subset of mutations is preferentially fixed during phenotypic transitions. Addressing this hypothesis requires comparing the genetic and developmental basis for convergent phenotypes at multiple evolutionary timescales, e.g. within populations, between populations, and between species. Flower color offers an ideal system for testing this hypothesis as this trait presents significant variation both within and between species and the underlying pigment pathways are well-characterized. I will examine the evidence for preferential fixation in Iochrominae, a florally diverse clade in the tomato family, Solanaceae. Results from our studies suggest that a range of coding and regulatory mutations are responsible for within species variation while only regulatory changes are fixed to give rise to species differences.

14.30-15.30 Poster Session (Posters P001-P179; see pages 50-143 of this booklet) & BREAK

Ginkgo Courtyard

15.30-17.30 Plenary Talks Session 10

(*Chair: Angelika Stollework, Queen Mary University of London)*

Krutch Theater

15.30-16.00 Ralf Sommer (Max Planck Institute for Developmental Biology, Tuebingen)

Molecular aspects of developmental plasticity: On novel genes, chromatin remodeling and developmental switch mechanisms

Ever since Darwin, biologists are intrigued about evolution and its underlying mechanisms. Two of the most astonishing aspects of evolution are diversity and novelty, but molecular mechanisms underlying these patterns are little understood. Developmental (phenotypic) plasticity - a widespread phenomenon in animals and plants - has been suggested to facilitate phenotypic diversity and novelty, and recent studies start to provide insight into associated molecular mechanisms. The nematode *Pristionchus pacificus* is a laboratory model for comparative mechanistic biology and shows phenotypic plasticity in its feeding structures by developing teeth-like denticles of two different forms. One mouth-form allows bacterial feeding, whereas the other one permits predatory feeding on nematodes and fungi (Bento et al., 2010). We analyzed the feeding dimorphism in *Pristionchus* nematodes by integrating developmental genetics with functional tests in divergent populations and species. We identified a regulator of plasticity, *eud-1*, that acts in a developmental switch (Ragsdale et al., 2013). Mutations in *eud-1* eliminate one mouth form, whereas over-expression of *eud-1* fixes this form. EUD-1 is a sulfatase that acts dosage-dependently and is sufficient to control the sexual dimorphism and micro- and macroevolutionary variation of feeding forms. EUD-1 is epistatic to known signaling cascades and results from lineage-specific gene duplications. More recent work indicates that *eud-1* is the primary locus of regulation of the mouth dimorphism with a dominant role of chromatin remodeling and the involvement of non-coding RNAs.

The existence of predatory feeding in nematodes ultimately results in the question of potential cannibalism. We have started to investigate this problem and identified what seems to be the first example of self-recognition in nematodes. While *P. pacificus* feeds on other nematodes, including other *Pristionchus* species and even the sister species *P. exspectatus*, it will not feed on conspecific larvae. I will report on the attempts to identify the underlying molecular mechanism of self-recognition. Finally, we tested how the new

predatory behavior and self -recognition are incorporated into an already existing nervous system. We show that *P. pacificus* employed a rewiring of the pharyngeal nervous system rather than the invention of novel cells during the transition from bacteriovorous to predatory feeding (Bumbarger et al., 2013).

16.00-16.30 Vivian Irish (Yale University)

A thorny question: Evolution and development of a novel mode of plant stem cell arrest

In flowering plants, the shoot apical meristem (SAM) consists of a few hundred stem cells that ultimately give rise to all shoot structures. This stem cell population continues to proliferate throughout vegetative development, forming leaves and branches in an indeterminate fashion. In contrast, thorns arise from vegetative SAMs that, instead of maintaining a stem cell fate, switch from indeterminate to determinate growth, resulting in terminal differentiation. Thorns appear to have arisen multiple times during angiosperm evolution, and are thought to have evolved as a protection against herbivory. Using *Citrus* as a model system, we are exploring the molecular mechanisms controlling stem cell proliferation and arrest in thorns. *Citrus* thorn meristems continue mitotic activity for a period of time, but relatively quickly cease divisions, their cells elongate and differentiate and eventually become lignified. In addition, *Citrus* thorn primordia express *STM*, a marker of meristematic fate. Using transient and stable transgenic methods, we are exploring the regulation of stem cell arrest in *Citrus* thorns using a candidate gene approach. We are also taking an unbiased transcriptomic approach to identify genes whose expression is correlated with thorn development. Together, these investigations should begin to define whether thorn development relies on a novel mode of stem cell arrest, or represents a redeployment of an ancestral mechanism to restrict stem cell proliferation.

16.30-17.00 BREAK

17.00-17.30 Matt Gibson (Stowers Institute)

Understanding animal origins: Establishment of the primary embryonic epithelium in the sea anenome, *Nematostella vectensis*

A crucial step in the evolution of multicellular animals was the organization of cells into the polarized and adherent monolayers known as epithelia. In this talk, I will describe novel TALEN and CRISPR/Cas9-based tools we have developed to genetically interrogate developmental mechanisms in a representative early-branching metazoan, the sea anemone *Nematostella vectensis.* I will also report on recent studies of early development, where cleavage-stage *Nematostella* embryos show initial signs of primary epithelialization between the 8- and 16-cell stage. During the

subsequent synchronous division cycles prior to the maternal-zygotic transition, we find that the structural integrity of the primary embryonic epithelium requires cell cycle-entrained oscillations of apico-basal polarity and cell-cell adhesion. Intriguingly, we detect similar oscillations during mitosis in mature epithelia of the fruit fly *Drosophila melanogaster,* perhaps consistent with broad conservation of mitotic polarity modulation throughout animals. Taken together, these experiments have implications for our understanding of epithelial cell division in general, and also provide conceptual insight into the conditions required for the evolution of multicellularity.

17.30-17.45 Poster & Talk Awards
(*Ehab Abouheif, President, McGill University*)

17.45-19.15 DINNER
Dining Center

19.15-21.00 Award Lectures
(*Chair: Karen Sears as President-Elect, University of Illinois at Urbana-Champaign).* Pioneer and Early Career Awards are sponsored by JEZB: Molecular Developmental Evolution.
Krutch Theater

Introduction by Greg Wray, Duke University
Rudy Raff (Indiana University)
Pioneer Award Lecture **Evo Devo in deep time: Embryos, genes and fossils**
The rapid evolution of embryonic development poses the puzzle of how the earliest steps of development can evolve despite lying deep in the processes that are the foundations for later development. Changes in patterns of early gene expression should be rare, but they are not. We have used two closely related sea urchins, *Heliocidaris tuberculata* and *H. erythrogramma*, that diverged about 4 MYA. These differ profoundly in early development. The first develops via feeding pluteus larva, but its congener omits the feeding larva and develops directly and rapidly into a juvenile sea urchin. Comparative single gene studies as well as transcriptome studies show that dramatic heterochronies in timing of gene expression, as well as shifts in the expression sites of some genes, are prevalent. Hybrids between the two species show the extreme robustness of early development to perturbations in gene expression when *H. erythrogramma* eggs are fertilized by *H. tuberculata* sperm, but early developmental failure in the reciprocal cross. Yet, comparisons with a sea urchin species (*Clypeaster rosaceus*), that is intermediate between indirect and direct development, show that early heterochronies can take place among developmental modules of indirect developers. The final part of the talk explores the ways in

which H. *erythrogramma* embryos illuminate how some of the earliest metazoan embryos entered the Precambrian and Cambrian fossil record.

Introduction by Elena Kramer, Harvard University
Natalie Pabon-Mora (Universidad de Antioquia)
Early Career Award Lecture **The evolution of floral and fruit developmental genetic networks**
Two genetic models have driven most of the research questions in plant evolutionary developmental biology for more than two decades. The first one, the ABCE model, postulates the overlapping expression and function of four classes of transcription factors that control floral organ identity. The second one, the fruit genetic network, describes an antagonistic relation between transcription factors involved in growth and histogenesis in dry dehiscent fruits. The two models were mainly based on molecular genetic analyses in the model species *Arabidopsis thaliana* (Arabidopsis). However, to understand the molecular basis of angiosperm morphological diversity, broad comparative studies are needed. Complications arise when attempting to identify functional homologs from the models due to the occurrence of whole genome duplications (WGD) during plant diversification. Thus, key to understanding the evolution of angiosperm synapomorphies is the inclusion of early diverging taxa, as they exhibit unique, likely ancestral, phenotypes and have not suffered all the WGDs that occurred during the radiation of monocots and eudicots. This talk addresses two case studies that test these models in a broad taxonomic range. The first case tests the ABCE model in Aristolochiaceae, a basal angiosperm family with a distinct floral groundplan: petaloid sepals, no petals and a congenital stamen-carpel fusion. The second case investigates the evolution of gene lineages involved in fruit development, and gathers expression and functional data from a diverse range of angiosperms. As a result, we hypothesize which genes and interactions from the Arabidopsis fruit development network might be important in shaping fruit morphological diversity.

21.00-22.00 Closing Reception
Garden Room

POSTERS

P001 Ehab Abouheif, McGill University

Past climate change on Sky Islands drives novelty in a core developmental gene network and its phenotype

A fundamental and enduring problem in evolutionary biology is to understand how populations differentiate in the wild, yet little is known about what role organismal development plays in this process. Organismal development integrates environmental inputs with the action of gene regulatory networks to generate the phenotype. Core developmental gene networks have been highly conserved for millions of years across all animals, and therefore, organismal development may bias variation available for selection to work on. Biased variation may facilitate repeatable phenotypic responses when exposed to similar environmental inputs and ecological changes. To gain a more complete understanding of population differentiation in the wild, we integrated evolutionary developmental biology with population genetics, morphology, paleoecology and ecology. This integration was made possible by studying how populations of the ant species *Monomorium emersoni* respond to climatic and ecological changes across five 'Sky Islands' in Arizona, which are mountain ranges separated by vast 'seas' of desert. Sky Islands represent a replicated natural experiment allowing us to determine how repeatable is the response of *M. emersoni* populations to climate and ecological changes at the phenotypic, developmental, and gene network levels. We show that a core developmental gene network and its phenotype has kept pace with ecological and climate change on each Sky Island over the last ~90,000 years before present (BP). This response has produced two types of evolutionary change within an ant species: one type is unpredictable and contingent on the pattern of isolation of Sky Island populations by climate warming, resulting in slight changes in gene expression, organ growth, and morphology. The other type is predictable and deterministic, resulting in the repeated evolution of a novel wingless queen phenotype and its underlying gene network in response to habitat changes induced by climate warming. Our findings reveal dynamics of developmental gene network evolution in wild populations. This holds important implications: (1) for understanding how phenotypic novelty is generated in the wild; (2) for providing a possible bridge between micro- and macroevolution; and (3) for understanding how development mediates the response of organisms to past, and potentially, future climate change.

P002 Rachel Agoglia, Stanford University

Disentangling sources of selection on exonic transcriptional enhancers

In addition to coding for proteins, exons can also impact transcription by encoding regulatory elements such as enhancers. It has been debated whether such features confer heightened selective constraint, or evolve neutrally. We have addressed this question by developing a new approach to disentangle the sources of selection acting on exonic enhancers, in which we model the evolutionary rates of every possible

substitution as a function of their effects on both protein sequence and enhancer activity. In three exonic enhancers, we found no significant association between evolutionary rates and effects on enhancer activity. This suggests that despite having biochemical activity, these exonic enhancers have no detectable selective constraint, and thus are unlikely to play a major role in protein evolution.

P003 Avery Andrus, California State University, Long Beach
Characterization of the innate immune response in *Patiria miniata* in comparison with other echinoderms
The complex immune systems of echinoderms have yet to be fully understood. Several types of coelomocytes (immune cells) are contained in the coelomic fluid of the water-vascular circulatory system. Phagocytic cells consume potential pathogens, and the coelomic fluid contains anti-microbial peptides, cell clumping factors, and other defense molecules. Most studies in echinoderms have been carried out in sea urchins. However, recently many species of sea star have contracted the Sea Star Wasting Syndrome, an infection characterized by the formation of white skin lesions, drooping, and tissue disintegration until death. A virus is associated with the symptoms, but the mechanisms and cause of tissue degradation are unknown. We have begun characterization of the innate immune system in the bat star Patiria minata in order to understand its role in the syndrome. We have characterized the coelomocytes in this species and examined their response to bacteria. We have also examined the proteins secreted into coelomic fluid that may play a role in immune function. We compare our findings to what is known in sea urchins, and have begun characterizing the coelomocytes in other echinoderm groups. A comparative study will provide information about the evolution of innate immunity in echinoderms. It may also help us understand why some echinoderms and not others are susceptible to the wasting syndrome. With new knowledge of echinoderm defensive pathways, it may be possible to increase the echinoderm defense against the virus, or prevent future large-scale mortality events.

P004 David Angelini, Colby College
The role of insulin signaling in development of wing morphs in the soapberry bug, *Jadera haematoloma*
Polyphenic traits develop different final states due to environmental cues. However, it is unclear how developmental processes differ to achieve distinct morphs. The red-shouldered soapberry bug Jadera haematoloma exhibits polyphenic wing morphs in both sexes, where adults may develop with complete wings and functional flight muscles or brachypterous wings incapable of flight. This system presents a combination of alternative patterning and growth, especially within the distal membrane region of the wing. We have explored the biology of the polyphenism using studies of growth, endocrine manipulations, and functional genetic tests using RNA interference. Wing morphs are determined are by juvenile food availability. Distal-less is required for growth within the distal region of the wing, which is reduced in short-wing morphs. Knock down of the insulin signaling pathway component

encoded by FoxO alters wing morph specification and results in significantly more short-wing individuals. Overall body size and wing allometry require insulin signaling. Knock down of the insulin receptor (InR) by RNAi caused smaller body size. Most appendages maintained normal allometry, scaled proportionally to body size, but wing lengths of J. haematoloma did not change under InR RNAi. Similar results were obtained from a related species without wing polyphenism, the milkweed bug Oncopeltus fasciatus, except that in this species wings scaled with body size after InR RNAi. These findings suggest that insulin signaling mediates growth in true bugs, and that polyphenism in J. haematoloma involves organ-specific modification of the insulin-signaling pathway.

P005 Shahaf Armon, Stanford University

Ultra-Fast Epithelium Contractions in a basal metazoan

Acto-myosin based epithelial contractions play a major role in tissue morphogenesis (for example gastrulation) and self-healing (wound repair) demonstrated by sheets of epithelial cells. The precise spatio-temporal control of contractions has long been perceived as the basic underlying mechanism behind coordinated transformations that large numbers of cells are capable of undertaking. These transformations are a collective phenomena where some prior information (for example cell polarity in case of tissue elongation) is pre-programmed while force feedback is used to tightly coordinate cell movement. Here we explore epithelial contractions in a primitive basal metazoan Trichoplax adhaerens (Phylum Placozoa), that has no known cell polarity in the plane of the epithelial sheet. By developing live cell labeling techniques and large field of view microscopy, we show here that dorsal epithelial cells in this organism demonstrate ultra-fast cellular contractions and expansion cycles (~40% reduction in area in 1 sec) over the entire adult life-cycle of the organism. A simple comparative analysis among other metazoans reveals that these cellular contractions are one order of magnitude (10 times) faster than cellular contractions seen broadly in Ascidian, Fly, Frog or mice embryos. Here we also study the asynchronous nature of these cellular contractions and how a comparison to simple coupled mechanical oscillators leads to the origin of domains and local synchronicity, as observed in live organisms.

P006 Deanna Arsala, University of Illinois at Chicago

A transcriptomic and functional approach to the regulation of early zygotic gene expression in haplodiploid embryos

In insects tissue fates are specified along the major axes, while at the same time the sex of the embryo is established through cell autonomous mechanisms in the blastoderm stage prior to gastrulation. Both of these processes are highly dependent on the proper transfer of maternal control to zygotic control during the MZT (maternal to zygotic transition). *Nasonia* wasps follow a haplodiploid sex determination system, with fertilized eggs resulting diploid females and unfertilized eggs yielding haploid males. However, sex identity is not directly dependent upon the ploidy but rather on levels of the female-specific splice-form of *Nv-transformer*. We are functionally testing differentially

expressed transcripts that are sexually regulated in the blastoderm stage in order to understand how sex identity is maintained zygotically. One particular transcript lacking identifiable domains and homologs outside of *Nasonia* is specifically up-regulated in female embryos at the late blastoderm stage. When it is knocked down through parental RNAi, female offspring that show marker gene phenotypes and patterns of inheritance that are consistent with being haploid. We hypothesize that this gene is necessary to maintain the presence of the paternal chromosome set in diploid females. We are also interested in how the MZT operates in haplodiploid organisms to establish sex identity and maintain proper development. We will characterize the *Nasonia* MZT using RNA-seq on *Nasonia* hybrids and functionally analyzing homologs of the two major players that have been identified in the MZT of *Drosophila, smaug* and *zelda.*

P007 Guillaume Balavoine, CNRS
Comparing segment formation mechanisms accross metazoans
WITHDRAWN

P008 Kenneth Baughman, Okinawa Institute of Science and Technology
Analysis of the Corallivorous Starfish *Acanthaster planci* genome reveals conservation between Echinoderms and Chordates
Acanthaster planci (Common name: Crown-of-thorn Starfish) is a corallivorous starfish, known for its consumption of hard corals in the Pacific and Indian Oceans. We sequenced, assembled, and annotated two draft genomes from two individual COTS starfish, in parallel. One specimen was collected on the Great Barrier Reef of Australia, the second specimen was collected at Motobu, Okinawa, Japan. Based on illumina short read sequencing data, total lengths of the respective genome assemblies are; Australia: 383,525,304 bp (N50 = 916,880 bp, 3274 total scaffolds) Okinawa: 383,843,944 bp (N50 = 1,521,119 bp, 1765 total scaffolds). The genomes align to each other with 98.7% identity. The A. planci Nkx cluster shows microsynteny with chordate Nkx clusters, which is consistent with collinearity we perviously reported within the starfish Hox cluster. 1-MA signaling signaling in oocyte meiotic resumption is unique to starfish among echinoderms, yet downstream components of this pathway are conserved across deuterostomes. In order to visualize these interactions, the components of the 1-MA pathway were identified from the literature, modeled in Systems Biology Graphical Notation (SBGN) diagram, and candidate transcripts and gene models were identified in the draft genome.

P009 David Baum, University of Wisconsin-Madison
The role of selection in shaping developmental-causal maps
A “phene” is a phenotypic feature that is dependent on a developmental-causal (DC) gene, whereas a DC gene is one or multiple genetic factors that cause a phene. I have previously argued that because phenes are caused by specific pieces of genetic information they are the only phenotypic features that can be seen to have homology relations (doi.org/10.3998/ptb.6959004.0005.003). This framework not only explains trait individuation in objective terms, but

allows for a definition of homology that does not depend upon the perceived similarity of characters in different organisms. As a result the phene concept has great potential for helping us think clearly about developmental evolution, providing fresh perspectives on serial "homology" (not really homology), developmental constraint, and the role of selection in shaping genotype-phenotype maps. I will briefly review the developmental-causal framework and then focus on the question, How does selection tend to shape the boundaries of phenes? I will hypothesize that when a phenotypic feature is important for fitness, selection will tend to turn that trait into a phene (if it is not already). Selection for robustness will also tend to reduce overlap among phenes, because each overlapping phene represents a potential mutation that could remove some or all of the selected phene. Finally, I will explore the idea that, insofar as DC genes function as identity determinants, selection for evolvability will also reduce phene overlap. While there remain many problems, this analysis provides a step towards articulating the role of selection in the evolution of development.

P010 Benjamin Blackman, University of Virginia

Shifting thresholds: The evolution of developmental plasticity to seasonal cues in Mimulus

Diverse taxa have adapted to use day length as an environmental cue for promoting the onset of reproduction because it serves as a reliable indicator of seasonal timing. Indeed, many plants only initiate floral development when photoperiod exceeds a critical minimum day length. Across latitudinal or elevational gradients, the timing of the growth season shifts, resulting in spatially varying selective regimes that maintain variation in flowering time and its plasticity to photoperiod. We have investigated the geographic diversity of critical photoperiod, flowering time, and their genetic basis in annual populations of the common monkeyflower Mimulus guttatus, an obligate long-day plant. Although critical photoperiod is strongly correlated with elevation and growth season start date, flowering time in inductive conditions is correlated with season duration and its relation to elevation changes with latitude. That these two components of an integrated seasonal phenology can separately evolve not only indicates that different agents of selection drive their evolution, but also predicts that unique genetic architectures govern divergence of each trait. Genetic mapping by multiplex shotgun genotyping in multiple high x low elevation crosses confirms this prediction: critical photoperiod loci do not co-localize with flowering time loci. Surprisingly, the loci contributing to clinal divergence in critical photoperiod are nearly all transect-specific, in contrast to many recent demonstrations of convergence at the genetic level and contradicting a classic prediction about how plasticity evolves. Finally, molecular studies in this system implicate copy number variation as a major contributor to geographic divergence in developmental plasticity.

P011 Julia Boughner, University of Saskatchewan

Red in Tooth and Jaw: Mechanisms coordinating the evo-devo of the mammalian face

To eat, hunt, vocalize and ultimately survive long enough to raise offspring, it is crucial to have teeth and jaws that develop properly in the right place, at the right time, relative to each other. The developmental-genetic processes that coordinate tooth-jaw growth remain elusive, yet discovering these mechanisms is important to understand how craniodental phenotypes properly develop and successfully evolve. With this aim, we have integrated molecular and morphological data from gene and protein expression, 3D imaging, geometric morphometric and anatomical network analyses of mouse and primate tissues to tackle the Evo-Devo biology of coordinated tooth and jaw formation. Using the "toothless" p63 mouse model aged embryonic days (E)10-13, we have identified a putative tooth-exclusive signaling pathway that is autonomous from lower if not also upper jaw skeletal development. Using rodent and primate specimens, we also deciphered for the first time new functional versus developmental and evolutionary links and dependencies among masticatory bones and muscles. Together these genetic, skeletal and muscular data suggest that development carries a stronger signal than function and is thus a major influence on craniodental evolutionary morphology; however, functional integration in the absence of pleiotropy appears to be the mechanism enforcing coordinated evolutionary change between the jaw and its dentition.

P012 Ingo Braasch, University of Oregon

Fresh insights from an old fish: Spotted gar illuminates the genomic basis of vertebrate Evo-Devo

Spotted gar (*Lepisosteus oculatus*) – a ray-finned fish that diverged from teleost fish shortly before the teleost genome duplication (TGD) and one of Darwin's defining examples of 'living fossils' – provides connectivity among vertebrate genomes and informs the ancestry of vertebrate development. The TGD had major impact on teleost genome and gene function evolution. Furthermore, the earlier two vertebrate genome duplications (VGD1/2) complicate vertebrate gene family and gene function analyses. Following the 'genomic big bang' of genome duplications, lineage-specific genome reshuffling and loss of gene duplicates obscure the distinction of orthologs and paralogs across lineages and thereby hide the origin of vertebrate gene functions. Using a chromosome-level genome assembly we show that spotted gar has a genome representative of the bony vertebrate ancestor and retained many paralogs from VGD1/2 that were differentially lost in teleosts and lobefins (coelacanth, tetrapods). The gar genome facilitates the identification of *cis*-regulatory elements shared among bony vertebrates, revealing hidden orthology of regulatory elements that cannot be established by direct teleost-tetrapod comparisons. Using whole genome alignments of teleosts, gar, coelacanth, and tetrapods, we identify gains and losses of conserved non-coding elements (CNEs) during vertebrate evolution, which also enables genome-wide analysis of the role of sub- and neofunctionalization after the TGD. Finally, we rear spotted gar as a developmental model in the laboratory to

functionally test hypotheses about the origin of vertebrate gene activities without the confounding effects of the TGD. Spotted gar is thus a powerful new model to study the genomic foundation of vertebrate development and evolution.

P013 Federico Brown, Universidade de Sao Paulo

Developmental modularity and ncRNAs during the evolution of coloniality in ascidians

To understand how asexual modes of reproduction evolved in our own phylum, we compare pudding process and genomes across several species of ascidians. In the Styelidae, species live as colonies of well integrated and synchronously developing individuals within a common tunic, as social aggregates of discretely developing individuals, or solitary individuals. Colonial botryllids (i.e. Botryllus spp. and Botrylloides spp.) form individuals synchronously, and generally develop by evagination of the lateral epidermis of adults. In contrast, Symplegma brakenhielmi colonies presents intermediate traits between colonies and social aggregates. Colonies are well integrated but individuals develop asynchronously resembling social forms. Buds generally develop independently at extracorporeal vessels that connect the individuals of the colony. To show that S. brakenhielmi individuals show complete independence in development, we carried systemic bud or zooid removal in the colony and compared our results to previous observations in Botryllus schlosseri. Next, we studied hemocytes and analyzed proliferation in S. brakenhielmi to identify putative circulatory progenitor cells. To associate genome evolution to coloniality in the tunicates, we use available genomes of solitary and colonial tunicates to begin to predict possible ncRNAs putatively involved in asexual life histories. We compare genomes of available solitary tunicates Ciona spp., Molgula spp., and Oikopleura dioica to the colonial Botryllus schlosseri genome, and the draft genome of the colonial Didemnum vexillum. Our results support a modular evolution of individual integration and developmental synchrony in colonial tunicates, and raise new questions about ncRNA regulation in stem cell function of colonial marine chordates.

P014 Heather Bruce, University of California, Berkeley

The genetic basis of limb morphogenesis in the crustacean Parhyale hawaiensis

Hox genes are well known for their role in patterning regional and appendage identity in Bilaterians. However, they are only part of the equation. As transcription factors, Hox proteins mobilize hundreds of downstream genes in order to build diverse morphologies. But, since all Bilaterians use essentially the same set of Hox proteins, the crucible of animal diversity must lie in these downstream interactions. This downstream network of genes is largely unexplored. To shed light on this topic, I am using the appendages of the crustacean Parhyale hawaiensis as a model system. Arthropod appendages are serially homologous structures that are patterned by Hox genes. Crustaceans such as Parhyale are ideal for this study because they have a plethora of highly modified appendages for sensing, chewing, grasping, defense,

mating, walking, swimming, clinging, and so on, which allows for the comparison of many derived morphologies in a single individual. To investigate the genetic basis of appendage diversity in Parhyale, I have generated Illumina mRNA expression profiles from 5 different appendage types at each of 7 developmental time points throughout appendage morphogenesis. These were aligned to a Parhyale de novo transcriptome generated by Jessen Bredeson (Rokhsar Lab) and myself. I have compared expression profiles from different appendages and time points to identify a set of differentially expressed genes. I am in the process of confirming the expression patterns of these candidate genes by in situ hybridization. Candidate genes with interesting expression patterns will be functionally tested using CRISPR-Cas9 knockout.

P015 Riva Bruenn, University of California, Berkeley

The Fearful Symmetry of Flowers: Investigating the role of TCP and MYB transcription factors in establishing dorsal/ventral asymmetry in the Zingiberales, an order of tropical gingers

The transition from many planes of symmetry (actinomorphy) to one plane (zygomorphy) in flowers increases pollinator specificity, contributing to the diversification of plant lineages. CYCLOIDEA and DICHOTOMA (TCP transcription factors), and RADIALIS and DIVARICATA (MYB transcription factors) make up the core of a floral symmetry network known from Antirrhinum majus. This network may be responsible for the many shifts from actinomorphy to zygomorphy across angiosperms. We investigated the conservation of the symmetry network in the Zingiberales, a monocot order with diverse floral morphologies and family-specific organs contributing to zygomorphy. If TCPs and MYBs control symmetry in the Zingiberales, these transcription factors may have unique whorl and organ specific expression and/or function across the order. Preliminary results indicate multiple copies of CYCLOIDEA-like, RADIALIS-like, and DIVARICATA-like, with evidence for Zingiberales-specific duplications in CYCLOIDEA-like and DIVARICATA-like. We utilized floral organ transcriptomes to estimate expression of each recovered gene, producing data consistent with a role for TCPs and MYBs in floral symmetry in the Zingiberales, with independent roles for different gene copies. Our results support the conservation of the Antirrhinum symmetry network in a distant monocot lineage, and provide insight into the role of gene duplication in network modification.

P016 Thibaut Brunet, EMBL

The origin and evolution of bilaterian muscle cell types: A view from the annelid Platynereis dumerilii

Muscle cells mediate every movement of our life - whether related to locomotion, digestion, communication, or reproduction. Myocytes are universally present in bilaterian animals, but the ancestral bilaterian musculature has been little investigated. Here, we address this question by providing a detailed characterization of muscular cell types in the annelid worm Platynereis dumerilii by single-cell transcriptional profiling, immunohistochemistry, cell lineaging, live imaging and electron

microscopy. Platynereis belongs to Lophotrochozoa, the third, and so far least investigated, bilaterian clade. Our study suggested the existence of an ancient bilaterian duality between somatic and visceral muscles, reyling on conserved complements of mutually exclusive transcription factors. This duality built upon an ancient duplication of myosin-encoding genes, which has been the first step of a duplication of cellular modules and, finally, cell types. Additionally, we describe distinct subsets of somatic muscles in Platynereis, and discuss their implications for the origin of certain key features of the chordate musculature, such as the hypaxial and epaxial myotomes, as well as the notochord - which is contractile in amphioxus and might be related to the axial longitudinal muscles of annelids and other bilaterians.

P017 Patrick Burton, Wabash College

Investigating Mechanisms of Regeneration and Wnt Signaling in Nematostella using Illumina

Regeneration is a widespread mechanism of animal development yet, because most model systems possess limited regenerative abilities, it remains poorly understood. The cnidarian Nematostella vectensis is capable of complete bidirectional regeneration. The Canonical Wnt Signaling pathway plays a conserved role in patterning the primary axis of Metazoa during embryogenesis, including Nematostella. While the components of the pathway itself are conserved across animals, the downstream targets of the pathway remain poorly understood in most taxa. As an initial step to investigate the mechanism of regeneration in Nematostella, and identify canonical Wnt Signaling targets, we examined gene expression via Illumina sequencing in regenerating polyps. Regenerating Nematostella were exposed to alsterpaullone, a GSK3 inhibitor previously shown to promote ectopic oral development via canonical Wnt signaling in Nematostella. Alsterpaullone treated samples were compared to control polyps undergoing either oral or aboral regeneration at 24 and 48 hours. We have identified a set of genes whose expression levels are significantly different in alsterpaullone treated samples relative to controls. We have also identified a set of genes whose expression differs in oral versus aboral regeneration. BLAST analysis indicates that many of the genes identified by this research have no known homologs among metazoans. Our results suggest that high throughput sequencing utilizing protein inhibitors is an effective method for identifying conserved signaling pathway targets in non-model systems.

P018 Christopher Cameron, Universite de Montreal

An ambulation through ambulacrarian & chordate evolution: Gills are an acorn worm novelty

Several novel lines of evidence support the hypothesis that the ancestor to the chordates & echinoderms resembled a hemichordate acorn worm. Like chordates, acorn worms have a pharynx perforated with gill slits. Here I bring together evidence from functional morphology, development, comparative genomics and palaeontology that shed light on this ancient beast, to which we chordates owe our existence and evolutionary success. i) Comparative morphology of feeding

adaptations between acorn worms and invertebrate chordates suggests that this pharynx perforated with gill slits was used in filter feeding. ii) A comparative analysis of hemichordate genomes has identified a conserved genomic cluster of co-regulated transcription factor genes associated with the development of pharyngeal gill slits, showing an ancient regulatory linkage across deuterostomes for this significant morphological innovation. iii) Numerous gene novelties shared by hemichordates and chordates imply physiological, metabolic, and developmental specializations of the filter-feeding deuterostome ancestor, including some novelties plausibly acquired through horizontal gene transfer from marine microbes. iv) The fossil Spartobranchus tenuis from the Burgess Shale of British Columbia puts backwards the record of acorn worms, with well-developed gills, to the mid-Cambrian (505 mya). Several extinct echinoderm classes also possessed gills. The molecular clock calibrates the divergence of the hemichordate-echinoderms at 559 mya and the chordate lineage at 579 mya.

P019 Terrence Capellini, Harvard University

When Evolution Hurts: Height, Arthritis, and the Cis-Regulatory Architecture of GDF5

Evolutionary modifications to limb developmental programs underlie limb length diversity and thus appendage functionality in mammals. Within primates, specifically human populations, numerous genetic loci have been discovered that control variation in height via their presumed influence on long-bone skeletal growth. In all cases, the causal DNA base-pair mutations that mediate the process remain undiscovered. Genetic variants in the GDF5 locus are significantly associated with height reduction and osteoarthritis susceptibility in human populations, yet the causal mutations controlling these phenotypes are unknown. We surveyed GDF5 for regulatory sequences using a mouse transgenic approach, and identified separate enhancers controlling expression in synovial joints and the growing ends of long bones. Sequences in a large intergenic region located downstream of GDF5 are required for functional rescue of both joint and growth changes in GDF5 mutant mice. In this downstream region, we identified a novel growth enhancer (GRO1) that colocalizes with the peak signals of positive selection at GDF5, and harbors a SNP with dramatic allele frequency differences among human populations. An evolutionarily derived form of the GRO1 enhancer, which shows reduced functional activity both in vivo an in vitro, is rare in many African populations, but common in Europe and Asia, and also found in Neandertals and Denisovans. Our studies suggest that an ancient regulatory variant in the GDF5 GRO1 enhancer has been repeatedly selected in northern environments, and that past selection on growth phenotypes explains the high frequency of a GDF5 haplotype that also increases arthritis susceptibility in many human populations.

P020 Chun-Che Chang, National Taiwan University

Germline development in the pea aphid Acyrthosiphon pisum: Developmental plasticity and evolution

The pea aphid *Acyrthosiphon pisum*, a hemimetabolous hemipteran insect with abundant adaptive capacity in response to specific environmental cues, has proven an excellent model for the study of developmental plasticity. Its published genome, moreover, has made research on embryonic development at the molecular level more accessible. Recent studies suggest that there are two distinct developmental programs directing axis patterning in the asexual viviparous and sexual oviparous embryos. In the germline cell lineage, we also found differential expression of duplicated *piwi* and *ago3* genes occurred during the transition from asexual to sexual phases. This suggests that reprogramming of the Piwi-interacting RNA (piRNA) pathway, where Piwi and Ago3 proteins are involved, is required during the switch of reproduction cycles. Using an affinity-purified antibody against the aphid germline marker protein ApVas1, nevertheless, we identified the preformed germ plasm in the uncellularized egg chambers of both viviparous and oviparous embryos. This indicates that specification of germ cells, unlike piRNA machinery, does not display developmental plasticity in both asexual and sexual reproductive cycles in the pea aphid.

P021 Samridhi Chaturvedi, Utah State University

Genomic Insights on the Recent Evolution of Novel Host Use in the Melissa Blue Butterfly (*Lycaeides melissa*)

The factors that shape the evolution of animal diets remain poorly known. For herbivorous insects, the expectation has been that trade-offs exist, such that adaptation to one host plant reduces success on other potential hosts. We investigated the genomic basis of alternative host plant use in Melissa blue butterflies (Lycaeides melissa) by analysing genetic variation in natural and experimental butterfly populations. We showed that distinct Melissa blue butterfly populations have independently colonized alfalfa since the 1800s when this plant was introduced, and that these populations have adapted to this novel resource. We documented segregating polygenic variation within and among butterfly populations for performance on alfalfa, and showed that different instances of adaptation to alfalfa have occurred via selection on a mixture of the same and different genes. Genetic variants in transposable elements and those in or near genes with metal-ion binding function might be particularly important for host adaptation. We documented very few loci with genetic trade-offs that would inherently constrain diet breadth by preventing the optimization of performance across hosts. Instead most genetic variants that affected performance on one host had little to no effect on the other host.

P022 Javiera Chinga, Pontificia Universidad Catolica de Chile

Ontogenetic integration in *Schizanthus* (Solanaceae) flowers; understanding the regulatory role of development on the patterns of morphological integration

The flowers of the genus Schizanthus are strongly zygomorphic and bilabiate with a high diversity of pollinator syndromes. We studied the pattern of ontogenetic integration of 5 species in which previous studies have found that the pattern of morphological (static) integration of the corolla is related with the functionality of the petals in the pollination process. In the three bee-pollinated flowers one of the two petals of the lower lip or both have important pollination function and gets more integrated with the corolla while in the two moth-pollinated the lower lip lost its function and gets decoupled. We measured 6 traits on the bud (5 petals and 1 stamen) with which we calculate the corolla integration degree (INT) across the ontogeny and before (early phase) and after (late phase) the joint of the two lips. We found that the patterns of ontogenetic integration were more similar among the species at the early phase and it reflects its evolutionary relationships. The pattern of ontogenetic integration is congruent with the pattern of static integration in all the species but in different ontogenetic stages; for the bee-pollinated flowers the pattern appears in the early phase and in the moth pollinated species in the late phase. This evidence also shows that structures generated from the same primordium can or cannot be integrated –so one of this structures may be reduced while the other is not- as this two possible developmental programs have been selected during the morphological evolution of Schizanthus´s flower.

P023 D. Nathaniel Clarke, Stanford University

Evolution of the Cadherin-Catenin adhesion module: Conserved functions in the Starlet Sea Anemone, *Nematostella vectensis*

The evolution of cell-cell adhesion and the early innovation of epithelial tissues were intimately coupled to the evolution of animal multicellularity and body-plan complexity. Epithelia perform a wide range of key biological functions, from acting as an environmental barrier and partitioning organisms into discrete compartments, to coordinating morphogenesis during development. A defining feature of epithelia is an adhesion module that glues cells together, enables folding of sheets of cells, and specifies gene expression. The adhesion module common to bilaterian animals consists of Cadherin transmembrane proteins, and the adaptor and signaling proteins α- and β-Catenin. Importantly, this multi-functional module is conserved in all animal lineages, and potentially satisfies all requirements for multicellular adhesion and morphogenesis: cadherins enable adhesion between cells, β-Catenin signals to the nucleus to alter gene expression, and α-Catenin binds to the contractile actin-myosin cytoskeleton to organize multicellular architecture. We have a detailed mechanistic understanding of the component parts and functions of this adhesion module in bilaterian animals, but the early evolutionary history of the module is far less clear. Does this cadherin-catenin module represent a minimal set of adhesion proteins necessary for the coordination of cell-cell adhesion and linkage to the actin cytoskeleton, and are basic functions of the module shared

by all animals? To explore these questions, we examined functions of cadherin/catenin-related proteins in a non-bilaterian animal, the cnidarian Nematostella vectensis. Our results, based on in vitro biochemistry and direct in vivo observation, demonstrate that the basic physical interactions and sub-cellular localization of cadherin/catenin-related proteins are conserved outside of bilaterians.

P024 Courtney Clark-Hachtel, Miami University

Exploring the origin of insect wings through functional analysis of vestigial in various arthropod species

Despite accumulating efforts to unveil the origin of insect wings, it remains one of the principal mysteries in evolution. Currently, there are two prominent models regarding insect wing origin: one connecting the origin to the paranotal lobe and the other to the proximodorsal leg branch (exite). However, neither hypothesis has been able to surpass the other. To approach this conundrum, we focused our analysis on vestigial (vg), a critical wing gene initially identified in Drosophila. Despite the well-accepted view of vg as an essential wing gene, our investigation in the Tribolium beetle led to the identification of two wing serial homologs in the "wingless" first thoracic segment (T1). Intriguingly, these two T1 wing homologs may actually be homologous to the two proposed wing origins (paranotal lobes and exite-bearing proximal leg segments). Therefore, our findings suggest that the vg-dependent tissues in Trobolium T1 could be wing serial homologs present in a more ancestral state, thus providing compelling functional evidence for the dual origin of insect wings. We are currently testing this model by (i) analyzing the development of the Tribolium T1 wing serial homologs in more detail (ii) evaluating the presence of the T1 wing homologs in another beetle (a diving beetle, Thermonectus) and a hemimetabolous insect (a cockroach, Blattella) to determine the lineage specific nature of the vg-dependent tissues in the wingless segments and (iii) analyzing wing homologs in a non-winged arthropod (Parhyale) to gain further insights into insect wing evolution.

P025 Lorna Cohen, University of Illinois at Chicago

Dissecting the genetic basis of head morphology and evolution in Nasonia

Identifying and characterizing the molecular basis of differences in form among species is one of the major goals of Evo-Devo. To address this, we are investigating the evolution of head morphology to unravel developmental differences among species in the *Nasonia* genus of parasitic wasps. The *Nasonia* genus is an emerging model clade quite apt for studies in developmental genetics and molecular evolution due to their haplodiploid genetics and the ability for interspecies crosses that result in fertile hybrid offspring. Of the four species within the *Nasonia* genus, *N. vitripennis* and *N. giraulti* are most suitable for morphological analyses as the head shape of the males is distinct from the other species. Further, *Nasonia's* haplodiploidy becomes especially useful in the study of interspecies differences in male traits, since the haploid male progeny of hybrid females can be screened directly in the F2 generation. Hybrid crosses between these two species reveal negative

epistatic phenotypes that are not present in either of the wild type species. We have developed a method of high-throughput phenotyping for comparative structural analysis of head shape in both the wild type and hybrid species. We intend to use this information, along with future genotyping experiments, to map these epistatic interactions and gain better understanding of the genetic architecture of morphological evolution.

P026 Kai Conrads, University of Cologne

Fog is rising: A signaling pathway used for morphogenesis across insects

Understanding morphogenesis is crucial to our knowledge of biology. One of the best-studied signaling pathways regulating morphogenesis is the Drosophila Folded gastrulation (Fog) pathway. This pathway features many stereotypical signaling mechanisms including patterned induction of gene expression by transcription factors, G-protein coupled receptor signaling, and Rho signaling induced actomyosin network rearrangements. In the Drosophila embryo, Fog signaling is active in several morphogenetic events, including ventral mesoderm invagination during gastrulation. To understand how such a pathway evolved, a comparative approach is needed. However, it is thought that fog is not conserved outside the higher Diptera. In contrast, we have found that fog is rapidly evolving and is in fact present in the wasp Nasonia vitripennis. Nasonia is a member of the Hymenoptera, the most basally branching lineage within the Holometabola. Both Nasonia and Drosophila share a similar, but independently derived, mode of long-germ embryogenesis in which all segments are patterned prior to gastrulation. The gastrulation movements of Nasonia are, however, clearly distinct form those of Drosophila. In particular, the mesoderm is not internalized via a ventral furrow and the germband undergoes very little extension. In spite of these differences, the Fog pathway is crucial in Nasonia for correct morphogenesis during gastrulation, as in Drosophila. We are investigating the cellular functions of the Fog pathway in Nasonia by carrying out RNAi mediated knockdown of concertina, which codes for the Gα subunit downstream of Mist, Fog's receptor, and analyzing the subsequent phenotypes.

P027 Brian Counterman, Mississippi State University

The Joint Development Genetic Control of Pigmentary and Structural Color Patterns on Butterfly Wings

Color patterns are one of nature's most dynamically rich and important signals of communication among plants and animals. Many of the striking color signals in nature require a coordinated arrangement of both pigmentary and structurally-based colors. Traditionally, color producing pigments and structures are thought to result from distinct developmental pathways. Here, we test the prediction that selection for the coordinated development of pigmentary and structurally-based color patterns on butterfly wings may actually involve a shared development genetic control. Specifically, we build from recent studies that demonstrated Wnt ligands pre-pattern the development of melanin on nymphalid butterfly wings by conducting pharmaceutical manipulations

of pupal wing development of the dogface butterfly (Pieridae: Zerene cesonia). Zerene butterflies are characterized by a distinct yellow "dog's face" that contrasts with the melanic patterns on the distal and proximal edges of the wings. In addition, this yellow dogface region also has bright UV reflection in males. Heparin injections produced all black forewings in both sexes, however males required twice the dosage as females to induce a similar melanic response. More importantly, the UV pattern was nearly completely missing, even in the males that did not develop black in the dogface region. The missing UV pattern appears to result from a lack of UV-producing nanostructures on the lamellar ridges of the wing scales. Collectively, these results suggest wnt ligands may be involved in the development of melanic patterns across distantly related butterfly lineages, and that these ligands may jointly regulate both pigment and structural color pattern development.

P028 Michael Czerwinski, Duke University

Transcriptome wide analysis of temperature dependent sex determination in the red-eared slider turtle *Trachemys scripta elegans* embryo

The study of temperature dependent sex determination (TSD) has long suffered from the difficulties inherent to restriction to non-model organisms. Many recent advances in the field of genetic sex determination have been made through system level approaches, made possible by large 'omic data sets that can be collected and analyzed with relative ease in these systems. With the recent rise in efficiency and accuracy of de novo transcript assembly and decline in the cost of next-gen sequencing, it is now possible to start applying these same system wide analyses to the study of TSD, even in non-model organisms. Here we present a transcriptome wide analysis of TSD in the red-eared slider turtle Trachemys scripta elegans. Through de novo transcript assembly and homolog based annotation from gonad tissue samples at the male and female producing temperatures we generated a transcript database for the assessment of gene and transcript level differential expression. We collected gonad samples from male and female producing temperatures across the developmental timecourse of the TSD period. Using a Hidden Markov Model (HMM) based clustering approach we identified multiple significant patterns of gene expression for comparison to similar data from the mouse genetic sex determination (GSD) system.

P029 Jacob Daane, Harvard Medical School

Phylogenomic evidence for parallelism and correlative selection during fish skeletal evolution

The identification of the genetic mechanisms underlying evolutionary change is critical to our understanding of natural diversity, but is presently limited by the lack of genetic resources for most species. We present a new comparative genomic approach, Phylomapping, that can be applied to a broad taxonomic sampling of non-model species to investigate the genetic basis of evolutionary change. Through enrichment of coding sequence by cross-species exome sequencing and the derivation of a shared 'ancestral' exome representing the

groups under study, the pipeline enables systematic analysis of genetic variation and patterns of selection between species lacking prior genetic resources. To examine the utility of this approach, we analyzed skeletal evolution within a lineage of cypriniform fishes for which no previous genomic or genetic resources were available. Using our analysis pipeline, we show that duplication and divergence of fgfr1a is correlated with the reduction of scales within fishes of the Phoxinellus genus, a defining trait for this group. We further identified fixed variation in fgf20a within Phoxinellus and demonstrate that combinatorial loss-of-function of fgfr1a and fgf20a in zebrafish is sufficient to phenocopy the evolved scalation pattern. Further, we used the capability of our exome-wide dataset to find systematic patterns of variation that may reflect causes of selection. Surprisingly, we observed broad patterns of selection on behavior-associated genes specifically within Phoxinellus. As reduced fgfr1 function also leads to aggression and boldness phenotypes in zebrafish and carp, these findings provide evidence for correlated trait evolution through pleiotropy as an important factor shaping morphological evolution.

P030 Gregory Davis, Bryn Mawr

Induction of reproductive fate in the pea aphid

The pea aphid, Acyrthosiphon pisum, exhibits several environmentally cued, discrete, alternate phenotypes (polyphenisms) during its life cycle. In the reproductive polyphenism, differences in day length determine whether mothers will produce daughters that reproduce either sexually by laying fertilized eggs (oviparous sexual reproduction), or asexually by allowing oocytes to complete embryogenesis within the mother without fertilization (viviparous parthenogenesis). Among other aspects of the polyphenism, we are interested in the process that specifies sexual versus asexual fate during embryonic development. Several lines of evidence implicate juvenile hormone (JH) in this process, namely that titers of JH correlate with day length (Ishikawa et al. 2012) and that topical application of JH can alter reproductive fate (Corbit and Hardie 1985). Together these observations suggest that high titers of JH are responsible for specifying asexual fate. We have explored this JH hypothesis further by testing whether JH is also required for the specification of asexual fate during embryonic development and attempting to discriminate among competing models for the role JH plays in the process. As a complementary approach we have used RNAseq to identify genes that are differentially expressed in sexually versus asexually fated embryos, both during the periods of specification and subsequent differentiation.

P031 Rui Diogo, Howard University

The end of an old dogma with crucial implications for evo-devo, medicine and the evolution of body plans: Regenerative, developmental, paleontological and evolutionary studies contradict the fore-hindlimb serial homology

Most developmental, evolutionary and medical textbooks state that the tetrapod fore and hindlimb are serial homologues. Here I show how regenerative studies of axolotls, developmental studies of salamanders,

frogs and amniotes, and comparative and evolutionary studies of all major vertebrate groups, including recent re-analyses of the appendicular muscles of chondrichthyans, dipnoans and coelacanths and work on human birth defects, contradict this old dogma. The integrative analysis of the data available strongly supports the idea that the similarity of the muscles and bones of the fore and hindlimbs of tetrapods such as salamanders and modern humans is not due to serial homology, but is instead the result of independent evolutionary changes (homoplasy) occurred mainly during the origin of tetrapods due to the co-option of similar genes for the development of both limbs (gene piracy). It also offers new insights about the ancestral Bauplan and morphogenetic gradients of tetrapod limbs, including the striking similarity of the zeugopodial (forearm/leg) and autopodial (hand/foot) muscles of the two limbs and ventro-dorsal symmetry of the zeugopodial muscles of a same limb, about the marked differences between pelvic and pectoral girdle structures, the homeotic transformations related to the number/identity of the digits and associated soft tissue changes, and the differences between limb ontogeny and regeneration, and about limb birth defects and their crucial medical implications.

P032 Rui Diogo, Howard University

Evolutionary Developmental Anthropology: A new field of science bringing together anatomy, human evolution, development, genetics, birth defects and medicine

Together with other colleagues, we are creating a new field of science: Evolutionary Developmental Anthropology. The main goal of this new field is to bring together anatomy, human evolution, development, genetics, birth defects and medicine, by using both non-human model organisms and studies of humans with birth defects or anatomical variations to address these issues. The combination of these fields and, importantly, the inclusion of both hard- and soft tissue-based information, allow us to address evolutionary and developmental questions that are not tractable using other types of studies and methodologies. Here we provide a few examples to illustrate the main aims and potential of this new field, focusing on: 1) the developmental and evolutionary origins of the human muscles; 2) notions of purpose and progress in evolution and the parallelism between ontogeny and phylogeny; 3) the relationship between trisomies, 'atavisms', evolutionary reversions and developmental constraints; 4) the tempo and mode of primate and human evolution, including discussions on modularity and ontogenetic constraints; 5) the relationship between modern human anomalies/variations, digit loss/gain, muscle changes and homeotic transformations; 6) similarities and differences among the hind and forelimb structures of modern humans; and 7) the variations and anomalies in the musculoskeletal system of modern humans and their evolutionary, developmental and medical implications.

P033 Seth Donoughe, Harvard University

High temporal resolution microscopy reveals nuclear dynamics of the insect blastoderm

In the vast majority of insect lineages, development begins with the formation of a syncytial blastoderm, yet the events that give rise to the uniform blastoderm are poorly understood, even in Drosophila. We use lightsheet microscopy to live-image transgenic embryos of the cricket Gryllus bimaculatus with high temporal resolution. We automatically detect and track nuclei, and then quantitatively characterize early divisions and movements of up to thousands of nuclei at a time in 3D space. Surprisingly, subsets of nuclei behave differently from one another even before the uniform blastoderm has formed. We have also used chemical disruptors of the cytoskeleton to probe the mechanistic basis of nuclear movements. Lastly, we have reconstructed the lineage relationship between extraembryonic tissue and the cells that form the embryonic rudiment. This has shed light on where and when embryonic cells are specified.

P034 Elizabeth Duncan, University of Otago

How do extracellular regulators of signalling pathways evolve?

Cell signalling pathways, such as BMP (bone morphogenetic protein) signalling, are used repeatedly in different contexts during development. The activity of these pathways must be exquisitely regulated to confine activity to the appropriate time and place during development. Extracellular regulators, such as Noggin, play an important role in this regulation. Noggin is a cysteine knot protein that was first characterised in the African clawed frog *Xenopus laevis,* where it has a crucial role in establishing dorsoventral polarity in the embryo by antagonising BMP signalling. We have recently shown that Noggin is related, at a sequence level, to the arthropod-specific proteins PTTH (prothoracicotropic hormone) and trunk. PTTH and trunk regulate metamorphosis and early embryogenesis, not through inhibition of BMP signalling, but through activation of MAP kinase signalling. We have also identified proteins very closely related to Noggin in a number of arthropod species including the pea aphid, *Acyrthosiphon pisum.* Using *Xenopus laevis* and *Drosophila melanogaster* we are assessing the function of our arthropod Noggin-like molecules. Based on the results of these functional assays we can make inferences and testable predictions about how these proteins have evolved from extracellular regulators of BMP signalling to activators of MAPK signalling.

P035 Ashley Duxbury, University of Georgia

Effects of diet and mating rate on germline stem cell turnover rate in the large milkweed bug, *Oncopeltus fasciatus* (Dallas)

Males can respond to the need for sperm. The mechanism by which this response is controlled has yet to be determined, but diet, and perhaps the number of mating partners, is thought to play a part. Using the large milkweed bug, *Oncopeltus fasciatus*, we explored the effects of diet and mating rate on sperm production and associated changes in fertility of males. Sperm production was estimated by the turnover rate of the germline stem cells (GSCs) while fertility was measured by counting

fertilized eggs laid by female mated with experimental males. Males were fed the ancestral diet of milkweed or the adapted diet of sunflower seeds, and subjected to a high or low mating rate. Dividing spermatocysts were stained using EdU and an antibody against phosphorylated histone H3. Our results suggest that diet does play a role in the amount of sperm a male produces, but mating rate does not. The milkweed fed males had higher GSC turnover at a younger age, while the sunflower fed males had higher GSC turnover at an older age. This agrees with earlier studies in our laboratory showing that milkweed fed males produce more offspring earlier in life and die earlier. Our results demonstrate how knowledge of developmental mechanisms can inform fundamental questions in the evolution of life history strategies.

P036 Allison Edgar, Duke University

A revised model for spider gastrulation

Spiders belong to the sister group of all other extant arthropod phyla. The longstanding canonical model of spider gastrulation, drawn primarily from one species, posits that cell internalization occurs only at a central blastopore; and that the cumulus (dorsal organizer) arises from within the deep layer by cell-cell interaction. Recent work has challenged this model, demonstrating spatial and temporal variation in cell internalization among species. We examined gastrulation in three species from two families. We traced individual cells from time-lapse recordings and examined the origin and fate of the cumulus by DiI labeling. We identified two distinct regions of internalization: the central blastopore and an additional extra-blastoporal ring, either at the edge of the germ disc (Latrodectus spp) or nearer the blastopore (Cheiracanthium). In all species, the cumulus cells internalized in concert through the blastopore and migrated away before other cells entered the deep layer. We propose a revised model of spider gastrulation. First, the prospective cumulus cells internalize and begin to migrate while other cell internalization pauses. This suggests cumulus specification is independent of interactions with other deep layer cells. Later, additional mesendoderm internalizes at two distinct locations: through the central blastopore and in a ring whose distance from the blastopore varies among our species. Our new model establishes a framework for future questions such as whether this latter site of internalization is common among spiders, whether the cells it internalizes are homologous across taxa, and whether its timing and location vary systematically.

P037 Lacey Ellington, Stowers Institute for Medical Research

TALEN and CRISPR/Cas9-mediated genome editing in the cnidarian *Nematostella vectensis*

TALEN and CRISPR/Cas9-based methods for genome editing have revolutionized research in model and non-model organisms alike, allowing the development of sophisticated gain- and loss-of-function genetic analyses. Here, we summarize our efforts to apply genome-editing tools in an emerging cnidarian model system, the starlet sea anemone *Nematostella vectensis*. Specifically, we will present homologous recombination-based strategies for mutagenesis and

transgenesis that should be applicable to other marine invertebrates with accessible embryos. Given its phylogenetic position as a representative out-group to the Bilateria, *Nematostella* has quickly become an excellent model for understanding the evolution of axial patterning, mesoderm induction, neurobiology and regeneration. The establishment of efficient genome editing methods in this system therefore has the potential to shed new mechanistic light on major questions in the evolutionary developmental biology of animals.

P038 Nick Ellis, University of California, Berkeley

Distinct developmental and genetic mechanisms underlie convergently evolved tooth gain in sticklebacks

When similar phenotypes convergently evolve in independent lineages, are the underlying genetic and developmental bases predictable? The threespine stickleback fish Gasterosteus aculeatus provides a system to address this question, as marine sticklebacks repeatedly adapt to countless new freshwater environments throughout the northern hemisphere. We found that two derived freshwater stickleback populations have convergently evolved more ventral pharyngeal teeth, likely an adaptation to a diet of larger prey in freshwater, through heritable genetic changes. In both populations, evolved tooth gain manifests late in development. Using pulse-chase vital dye labeling to mark newly forming teeth in adult fish, we find that both high-toothed freshwater populations have accelerated tooth replacement rates relative to low-toothed ancestral marine fish. Despite the similar evolved phenotype of more teeth and an accelerated adult replacement rate, the timing of tooth number divergence and the spatial patterns of newly formed adult teeth are different in the two populations, suggesting distinct developmental mechanisms. Using genome-wide linkage mapping in marine-freshwater F2 genetic crosses, we find that the genetic basis of evolved tooth gain in the two freshwater populations is largely distinct. Together our results support a model where increased tooth number and an accelerated tooth replacement rate have evolved convergently in two independently derived freshwater stickleback populations using largely distinct developmental and genetic mechanisms.

P039 Joakim Eriksson, University of Vienna

Has a new segment evolved in the head of the Onychophora?

Arthropod head segmentation remains elusive after a century of debate, however, now mainly focusing on the pregnathal area in front of the antennae. Recent molecular genetic characterization has shown that the most anterior area of the bilaterian head might be traced back to a common ancestor. This area corresponds to the anterior expression domains of the genes six3 and orthodenticle(otd) and is believed to form from a preoral unsegmented region of the bilaterian body. However, in onychophorans or velvet worms there is no obvious anterior part that can be attributed to any unsegmental region. In this investigation we show that the previously described six3-otd domain in onychophorans precisely corresponds to the anterior metameric unit in the onychophoran head. The expression pattern of pax6 and dachshund in

this anterior region further strengthens the homology of this region with anterior brain structures in other bilaterians. The presence of distinct segmental characters in this region suggests that a new segment evolved anteriorly in the onychophoran lineage.

P040 Priscilla Erickson, University of California, Berkeley

Functional genetic analysis of stickleback craniofacial evolution

Understanding the genetic and developmental basis of morphological diversity is a primary goal of evolutionary biology. The genomic resources and phenotypic diversity of the threespine stickleback system provide an excellent model to study the genetic basis of morphological evolution. Marine and freshwater sticklebacks are adapted to different diets and have striking differences in the food-processing structures of the branchial skeleton: freshwater fish have more teeth and longer branchial bones due to both early and late developmental differences. Overlapping quantitative trait loci (QTL) on chromosome 21 control these skeletal differences in multiple freshwater populations, suggesting either a single pleiotropic locus or closely linked loci control these traits. The tooth number QTL maps to a small genomic region containing a cis-regulatory allele of *Bmp6,* with the freshwater allele having reduced expression. A short, 190 bp upstream enhancer is required for *Bmp6* expression in teeth. While a TALEN-generated coding mutation in *Bmp6* reduces tooth number in heterozygotes and is lethal in homozygotes, fish heterozygous or homozygous for a loss-of-function mutation in the enhancer are viable and have more teeth, mirroring the evolved phenotype. However, recombinant chromosomes exclude the coding region of *Bmp6* from the bone length QTL. Ongoing studies are functionally testing the roles of *Bmp6*, and a nearby developmental regulatory gene, *TFAP2a,* in bone length evolution using loss- and gain-of-function approaches. Combined, these results suggest multiple tightly linked loci control different skeletal adaptations and that *cis*-regulatory changes can produce evolved phenotypes while avoiding deleterious pleiotropic effects.

P041 Timothy Evans, University of Arkansas

Axon guidance roles of Robo receptors in Drosophila and Tribolium

Roundabout (Robo) family axon guidance receptors regulate axon guidance in bilaterian animals, including midline crossing of axons and the formation of longitudinal axon pathways. The Robo family has expanded by gene duplication in insects and vertebrates, where individual receptors display unique sets of axon guidance activities. In Drosophila, Robo and Robo2 mediate midline repulsion in response to Slit, while Robo2 and Robo3 specify lateral position of longitudinal axon pathways. Alone among the fly Robos, Robo2 can also promote midline crossing by antagonizing canonical Slit-Robo repulsion. Drosophila robo2 and robo3 are products of a recent gene duplication, and Robo2's unique role in promoting midline crossing may be a recent evolutionary development. To gain insight into the evolution of axon guidance mechanisms in insects, we have begun to characterize the axon guidance roles of Robo receptors in the flour beetle Tribolium

castaneum, which unlike Drosophila has only two Robo genes: Robo (TcRobo) and the ancestor of Robo2 and Robo3 (TcRobo2/3). We have found that TcRobo2/3 can partially substitute for robo2 to promote midline repulsion and axon pathway formation in the Drosophila embryo, but is unable to rescue robo2's pro-crossing role, suggesting that the pro-crossing role of fly Robo2 is not conserved in Tribolium and may have been acquired by Robo2 after its divergence from Robo3. Our results provide insight into the evolution of axon guidance mechanisms, and reveal that modern insects deploy divergent genetic programs to control axon guidance decisions during development.

P042 Cera Fisher, University of Connecticut

Characterizing the evolution of novelty in treehoppers (Hemiptera: Membracidae) through a comparison of tissue-specific transcriptomes

Treehoppers (Hemiptera: Membracidae + Aetelionidae + Melizoderidae) are sap-sucking insects that fascinate naturalists due to their elaborate helmet, an enlarged dorsal projection of the pronotum exoskeleton that has been molded by natural selection into an array of structures aiding mimicry and crypsis. Members of their sister group, the leafhoppers (Cicadellidae), exhibit the plesiomorphic condition, having a short, collar-like pronotum. The developmental genetics and origin of the treehopper helmet, though hotly debated in recent years, remain a mystery. Evidence from gene expression in treehoppers and RNA interference in other insects suggest that co-option of canonically wing-patterning genes may be involved, while evidence from anatomically similar beetle horns suggest the possibility of leg-patterning gene co-option. To test these and other hypotheses, we apply an RNA-Seq approach to analyzing gene expression in four tissues of nymphal Entylia carinata (Membracidae) and Homalodisca vitripennis (Cicadellidae). Using multi-dimensional scaling and hierarchical clustering to characterize and compare the suites of genes involved in each tissue's development, we hope to shed light on the subject and produce candidate genes for further functional genomics work.

P043 Emily Fishman, University of Toledo

The Evolution of Atypical Centrioles during Animal Reproduction

Centrioles are conserved subcellular structures with 9-fold symmetric microtubules. Upon fertilization, the sperm centrioles form the zygote centrosome, which mediates the fusion of the male and female nuclei, producing genetically unique offspring. The centrioles of most animals originate from the father, but the identity of the paternal centrioles is debated, as often they are not visible due to a process called centrosome reduction, which occurs during spermiogenesis. During centrosome reduction, centrioles are dramatically modified, losing their 9-fold symmetric microtubules and centriolar proteins, leading to distinct hypothesis on centriole inheritance in various animals. To resolve that, we propose a universal hypothesis, in which the animal ancestor had two sperm centrioles, modified by centrosome reduction, but still functioned in the zygote. Later, during animal evolution, centrosome reduction was modified, producing sperm centrioles of varying structure

and composition. This hypothesis originated from our discovery of a novel second centriolar structure, the proximal centriole-like structure (PCL), in Drosophila sperm. Because of the PCL's similarities and differences to centrioles, we have deemed it an atypical centriole. Like typical centrioles, the PCL functions in the zygote, forming a centrosome that is essential for zygote division. However, unlike typical centrioles, the PCL is missing 9-fold symmetric microtubules, but is composed of centriole-specific proteins and is dependent on the same molecular pathway for formation. Preliminary data suggests that Tribolium has a similar second centriolar structure, supporting our hypothesis. We will discuss Drosophila's PCL, the second centriolar structure in Tribolium sperm, and our universal model for centriole inheritance.

P044 Rachel Flores, California State University, Long Beach
Identification and Characterization of Conserved Proteins in the Echinoderm Skeleton
Specification of the cell fate of skeleton-forming cells in developing sea urchin embryos has been well characterized and the skeletal proteome of both adult and larval skeleton has been determined. Little is known, however, of the mechanism and proteins involved in the mineralization process. A number of spicule matrix proteins have been identified in sea urchins and are present in the organic matrix during spicule formation. However, loss of function studies have indicated they may not be required for mineralization. We have taken a comparative, evolutionary approach to try and elucidate what proteins may be required for the process of mineralization. Here we report the characterization of the skeletal proteome of the sea star Patiria miniata. By comparing the proteins present in all mineralized tissues of the sea urchin with those found in other echinoderm groups we have identified a set of conserved proteins containing similar functional domains. The skeletal proteomes from the two echinoderm, P. miniata and Strongylocentrotus purpuratus, groups also shared novel proteins with similar functional domains. We have analyzed the sequences of the proteins we identified as potentially important to determine the evolutionary relationships and to identify the sequences most highly conserved. Using the sea urchin S. purpuratus as a model echinoderm, we have determined the temporal expression pattern of a number genes encoding conserved biomineralization proteins using quantitative PCR. Our comparative approach has allowed us to identify a set of conserved proteins whose role in biomineralization has not previously been examined.

P045 Vanessa Flores, University of California, Berkeley
Wnt signaling in the posterior growth zone of the leech *Helobdella austinensis*
Axial growth and patterning is a defining feature of bilaterian animals, and is modified to a segmented body plan in three highly successful phyla (annelids, arthropods and vertebrates). Leech (annelid) is useful for studying axial growth and segmentation within the relatively understudied super-phylum Lophotrochozoa. Leeches undergo lineage-driven segmentation, driven by a posterior growth zone (PGZ),

consisting of five pairs of lineage-restricted stem cells (teloblasts), and their progeny (blast cells). We are investigating Wnt signaling in PGZ formation and function, because: 1) this conserved signaling pathway functions in segmentation and axial growth across Metazoa; and 2) multiple *wnt* genes are expressed in the PGZ. Treating with LiCl to activate Wnt signaling just prior to teloblastogenesis disrupts this process. Lineage tracing shows that the ventralmost (N) teloblast makes an abnormal symmetric division in ~30% of embryos. We are using time-lapse microscopy to ask if this abnormal division occurs at a specific division during development, and if both teloblast-like cells produce blast cells. In other experiments, we are studying the roles of specific Wnt ligands in blast cell fate specification. *wnt16b* is expressed in a small subset of blast cells very early in the segmentation process; preliminary observations suggest that *wnt16b* is turning on and off in different lineages in a stereotyped manner. We are using experiments with combined lineage tracer and in situ hybridization to determine the specific spatiotemporal pattern of *wnt16b* expression, after which we hope to use antisense morpholino oligomer to elucidate its function in these cells.

P046 Rodrigo Fonseca, Universidade Federal do Rio de Janeiro
The pioneer transcription factor zelda is required for growth zone patterning and metamorphosis in the beetle *Tribolium castaneum*
Gene regulatory networks (GRN) result from the evolution of transcription factors and the cis-regulatory modules (CRMs) they bind to. The zinc-finger transcription factor zelda (zld) is essential for maternal zygotic transition (MZT) in Drosophila melanogaster, when it directly binds over thousand CRMs and regulates chromatin accessibility. Drosophila displays a long germ type of embryonic development, where all segments are simultaneously generated along the whole egg length. It remains unclear if zld is also involved in MZT of basal groups (e.g. short-germ insects) or in other biological processes. Here, we provide the first biochemical, morphological and computational analysis of zld in the short-germ beetle Tribolium castaneum (Tc). Computational analyses identified segmentation, metamorphosis and wing formation as putative new biological roles of zld in Tribolium. mRNA expression and knockdown during several developmental stages (embryo, larvae, pupae and adult) confirmed that Tc-zld is essential not only for MZT, but also for the aforementioned processes in short-germ insects. These results imply that the pioneer transcription factor zld is not only involved in MZT but also in other key developmental processes. We discuss the implications of these findings for the evolution of gene regulatory networks in arthropods.

P047 Nadine Frey, University of Cologne
Identification of new dorsoventral patterning genes by differential transcriptome analyses in *Tribolium castaneum*
The patterning of the dorsoventral (DV) axis of Drosophila is one of the best studied developmental processes. In Drosophila, dorsoventral patterning is dominated by Toll-signaling whereas in most non-insect bilaterian lineages BMP plays a major role in the establishment of the

DV axis. Within insects, representatives of more basally branching lineages show an increased reliance on BMP signaling while the importance of Toll signaling is reduced. However, our knowledge of how the DV gene regulatory network (GRN) changed during insect evolution is so far largely based on a candidate gene approach. To gain an unbiased global understanding of a DV GNR, we performed transcriptome analyses after RNAi in the short germ beetle Tribolium castaneum, an insect with more basal features of embryogenesis compared to Drosophila. We manipulated both Toll signaling, including the important Toll target gene twist, and BMP signaling. In order to identify differentially expressed genes, we compared the transcriptomic data of knockdown embryos from Tc-Toll, Tc-twi, Tc-short gastrulation and Tc-decapentaplegic to control samples. An in-situ hybridization screen was performed with differentially expressed genes to test for DV asymmetric expression during early Tribolium embryogenesis. Furthermore, genes with interesting expression patterns in blastoderm or germband stages were chosen for functional studies by means of pRNAi. Using this approach we were able to identify new components of the DV-GRN of Tribolium castaneum. Functional studies of the investigated candidate genes will lead to a better understanding of the evolution of the DV-GRN in insects.

P048 Jens Fritzenwanker, Stanford University

Axis elongation in the absence of segmentation: Hemichordate posterior growth and the evolution of the bilaterian trunk

Besides the head and a proximal portion of the anterior trunk, a large part of the bilaterian body commonly develops through a mechanism called posterior axis elongation. Since the so formed trunk makes up a substantial part of the animal body it plays a crucial role in animal evolution. However, much of what we understand about the developmental control of posterior axis elongation comes from studies of chordates and arthropods. In these groups posterior axis elongation encompasses two mechanisms; segmentation and growth which are tightly coupled making broad evolutionary comparisons of mechanisms relevant for posterior growth challenging. In order to gain a broader understanding of the process of posterior growth in deuterostomes, we have carried out a functional analysis of axis elongation in the hemichordate Saccoglossus kowalevskii. As an unsegmented phylum closely related to chordates, hemichordates offer a unique opportunity to investigate posterior axis elongation in the absence of segmentation. Elongation of the vertebrate trunk is regulated by a complex network of gene-regulatory feedback loops and interactions between signaling pathways. We have carried out a detailed functional analysis of two key components of vertebrate posterior axis elongation; canonical Wnt signaling and brachyury. We show that canonical Wnt signaling acts in a feedback loop with brachyury during posterior growth and that this feedback loop interacts with Notch and FGF signaling. We discuss the comparative implications of this work for understanding the early evolution of the bilaterian trunk.

P049 Daniel Fulop, University of California, Davis

Genotyping by heterogeneous HMM and epistatic QTL mapping by sparse regression: A case study in interspecific tomato leaf shape and complexity

QTL mapping is a powerful technique for dissecting the genetic basis of species differences and as such it is an essential part of the Evo-Devo toolbox. However, there are many limitations to QTL mapping, such as large QTL intervals encompassing many genes and methodological obstacles to revealing the genetic architecture of traits. We overcame these and other obstacles by developing our own statistical inference and visualization tools, and by using state of the art sparse regression techniques. We studied leaf complexity and leaflet shape in a large BC2/BC3 S. pennellii x S. lycopersicum population. To call genotype blocks from RADseq data we developed an HMM with a heterogeneous transition matrix, in which the transition probability depends on a function of the genetic distance between SNPs. The HMM inferred narrow transition boundaries in spite of noise and/or low coverage in some of the lines. A set of BINs were defined by merging nearby transition boundaries and identifying linkage blocks within which no line changes genotype. We used sparse regression to regress leaf traits on all BIN genotypes and all potential 2-BIN epistatic interactions simultaneously. Thus, we circumvented the need to condition the epistatic search to loci with a significant main effect. A set of literature-curated candidate leaf shape genes was enriched in the QTL of many traits, in part owing to the narrow QTL intervals inferred. This study demonstrates the power for mapping epistatic QTL provided by large back-crossed populations in combination with sparse regression.

P050 Eve Gazave, CNRS

Notch pathway involved in annelid bristle development but not early neurogenesis

Notch is a key signalling pathway shared by all metazoans. Its functions during development are multiple and varied. However, striking similarities have been uncovered between insects and vertebrates in the role Notch plays during neurogenesis. Here, we explore the functions of the Notch pathway in the embryos and larvae of the marine annelid Platynereis dumerilii, as a representative of the lophotrochozoan branch of the metazoan tree. We identified well conserved components of the pathway in the P. dumerilii genome and complete a scenario for the molecular evolution of this pathway. Unexpectedly, neither Notch nor its ligand Delta are expressed in the neurogenic epithelia of the larva at the time when neurogenesis begins. Using Notch small molecule inhibitors and specific neural markers, we demonstrate that the pathway plays no role in general larval neurogenesis. Instead, we find specific expressions of Notch components in the chaetal sacs, the organs that produce the characteristic chitinous bristles of the annelids. We show that impairing Notch signalling causes incorrect specification events in the chaetal sac and an absence of bristle growth. We describe the first case supported by experimental evidence of a bilaterian species in which the early neurogenesis processes occur without the involvement of the Notch pathway. This particular feature, together with its

demonstrated co-option in the process of chaetae formation, exemplifies the remarkable “toolkit” nature of the pathway. This work reinforces the view that Notch signalling has been recruited multiple times in evolution due to its molecular properties.

P051 Andrew Gehrke, University of Chicago

Ancient origins of the Hox regulatory system in vertebrate appendage development

Mammalian limbs are built by two sequential periods of Hox gene activation, commonly referred to as "early" and "late" phases that pattern proximal (arm) and distal (hand) segments, respectively. The regulatory systems responsible for the dual nature of Hox gene expression in limbs have been extensively studied in mouse, where early and late phase enhancers lie on opposite sides of the HoxD and HoxA clusters. Fish exhibit a similar pattern of Hox gene activation during fin development, but the extent to which these patterns are homologous with limbs remains controversial. Thus, identifying the extent to which the regulatory systems controlling Hox expression are conserved or divergent in fish remains crucial to understanding the development and evolution of appendages. Using multiple sequence alignments with key taxa combined with epigenomic profiling, we identified both “early” and “late” phase Hox enhancers in the genome of a variety of fish species. When tested in transgenic zebrafish, these regulatory elements drove reporter expression in pectoral fins reminiscent of the biphasic pattern in mouse limbs. Furthermore, when tested in transgenic mice, enhancers from specific fish were able to drive limb expression in an identical pattern to their murine orthologs. Altogether, these data suggest an ancient origin of the vertebrate Hox appendage developmental system, implying homology between the distal bones of fish fins and the wrist/digits of tetrapods. Thus, changes in fin morphology may have resulted from subtle modifications to this network rather than the evolution of novel expression domains.

P052 Ivan Gomez-Mestre, Donana Biological Station, CSIC

Canalization of developmental plasticity in spadefoot toads: From phylogenetics to transcriptomics

Developmental plasticity allows populations to withstand rapid environmental changes and confers a fast rate of adaptation. However, if plasticity costs are high and environmental variation is buffered, selection leads to genetic accommodation. Phenotypic divergence between species may thus initiate as environmentally-induced expression of alternative phenotypes. Descendant lineages of a plastic ancestor evolving in more stable divergent environments may lose plasticity over time. In that case, we would expect ancestral plasticity to mirror differences among taxa, and also that the same mechanisms regulating ancestral plasticity explain among-species divergence. In that light, we are studying mechanisms of plasticity behind the evolutionary divergence of spadefoot toads. Old World species (Pelobates) breed in long lasting ponds and have long but plastic larval periods, whereas New World species (Scaphiopus) have specialized in ephemeral ponds and have evolved very short larval periods. We hypothesize that

Scaphiopus has undergone genetic accommodation of ancestral plasticity, which has resulted in canalized short larval periods. To test this hypothesis we have studied the mechanisms underlying developmental acceleration in response to pond drying and compared it across species. We find that Pelobates tadpoles, which reflect the ancestral state of the group, increase their metabolic rate, and thyroid hormone and corticosterone concentrations in response to decreased water levels. All these parameters, however, seem to have been greatly canalized in Scaphiopus. Having sequenced and assembled the transcriptomes of these species we can now examine differential gene expression in response to pond drying testing for transcriptomic signatures of genetic accommodation.

P053 Philip Grayson, Harvard University

The developmental basis of convergent flight loss in paleognathous birds

Convergent evolution produces shared, analogous phenotypes along independent lineages. Through this process, pterosaurs, bats, birds, and groups of insects have gained flight, a complex event that requires many physiological and structural phenotypic changes to occur in tandem. Flight has also been lost across diverse avian taxa including parrots, wrens, pigeons, rails, penguins, ducks, grebes, and ratites. The palaeognaths, a clade including the flightless ratites and volant tinamous, offer a unique opportunity to study the processes underlying convergent evolution: recent phylogenies suggest that flight has been lost a minimum of three times within the clade, with some authors claiming more than six independent losses. Ratites have flat, raft-like keels (the extension of the sternum normally used to anchor flight muscle) and have all experienced forelimb reduction to varying degrees (reduced wings in ostrich and rhea, vestigial wings with digit loss in emu, kiwi and cassowary, and complete wing loss in moa). Both of these phenotypic modifications are common across flightless avian groups. Using an integrative approach involving the sequencing of ten new paleognath genomes (three tinamous and seven ratites) and developmental experiments in emu (Dromaius novaehollandiae), rhea (Rhea americana), and chicken (Gallus gallus), we are identifying and testing candidate genes and conserved noncoding regions with convergent evolutionary signals of gain, loss, or modification consistent with roles in the repeated loss of flight across paleognathae. Broadly, this approach allows us to examine the functional developmental basis of a common convergent phenotype.

P054 Stephen Green, California Institute of Technology

Deep conservation of a deuterostome gene regulatory network controlling 'cranial' mesoderm development

The origins of craniofacial structures, including distinct cranial muscles, are among the most important events of early vertebrate evolution. Analyses have shown that vertebrate cranial muscles arise from progenitor cells whose fate is directed by a gene regulatory network (GRN) that includes Tcf21 and Tbx1. This network is distinct from that present within the somitic myotomes. The origins of the cranial muscle

GRN are unclear, and the GRN might have arisen in early vertebrates as a component of the vertebrate 'new head.' To test this hypothesis, we examined the spatiotemporal expression patterns of homologs of cranial muscle genes identified in the direct-developing hemichordate Saccoglossus kowalevskii and the sea lamprey Petromyzon marinus. Both gene expression data and functional data suggest that hemichordates have a GRN controlling the formation of anterior mesoderm that is distinct from the GRN regulating hemichordate posterior mesoderm but similar to the GRN regulating vertebrate cranial muscle progenitors. This suggests that a distinction between cranial and somitic muscle did not originate in early vertebrates but instead may be derived from mesoderm patterning in early deuterostomes. These findings are important for understanding the evolution of the vertebrate head and the evolution of body patterning in early deuterostomes.

P055 Theresa Grieco, University of British Columbia

Evaluating biological causation in classical models of tooth replacement: A study of spatiotemporal patterns in the leopard gecko dentition

Many reptiles replace their teeth continuously, providing an opportunity to understand the regulation of tooth development within a dentition. As in mammals and humans, reptilian replacement follows a stereotypical sequence that has consequences for the overall functionality of the tooth row. To document this sequence in leopard geckos (Eublepharis macularius), upper jaw wax impressions were collected in a longitudinal study to mark tooth shedding events in hatchling to juvenile stages. The compiled dental arrays revealed dynamic patterns of tooth shedding and midline symmetry. Typically, a tooth is shed in one week and replaced by a functional tooth within the next week. When viewed as a spatial pattern across the tooth row over time, waves of tooth shedding pass through even and odd series from the back to the front of the jaw. Alternatively, these same waves could be viewed as linking adjacent tooth positions and passing from anterior to posterior in the jaw. The regularity of the observed patterns suggests emergent tooth turnover phenomena that may result from the order of embryonic tooth initiation, local activation and inhibitory influences within the jaw, and the rates of development within tooth families. Mathematical and biological models, including local inhibition and wave-stimulus models, have been proposed to explain the regular tooth replacement patterns observed in nature but the proposing authors had limited access to molecular and temporal data to support them. These theoretical models are reconsidered in light of temporal and spatial parameters and variants identified in leopard geckos.

P056 Sumant Grover, University of Dayton

Patterns in Evolution: Tracing the Genetic and Molecular Basis for a Convergent Fruit Fly Pigmentation Pattern

The genetic basis by which organisms adapt to an ever changing world remains a topic of great interest to the fields of evolution, development, and conservation biology. It is understood that animal genomes contain over ten thousand genes and distantly related species possess many of

the same genes due to common ancestry. What is less well understood is how new traits evolve using these shared genes and whether the genetic basis for evolution favors certain genes over others. At the heart of trait development are genes that encode proteins that regulate the expression of other genes, notably transcription factors and chromatin modifying proteins. Traits can evolve through changes in the expression patterns for these genes or through changes in which target genes they regulate. However, case studies connecting gene expression changes to trait evolution remain few in number. Additionally, it is unclear whether gene expression evolution favors alterations in certain genes over others. In order to understand how a novel trait evolves and to determine whether evolution can prefer certain gene targets for modification, we are studying the convergent evolution of fruit fly pigmentation in the lineages of *Drosophila melanogaster* and *Drosophila funebris*. These two species can be considered biological replicates for the evolution of male-specific pigmentation on the A5 and A6 abdominal segments. To understand the genes involved in the formation and evolution of these similar pigmentation patterns, we are utilizing candidate gene and comparative transcriptomic approaches. Completion of this work will provide novel insights on the genetic changes responsible for a trait's origin, and whether development constrains evolutionary paths to certain genes.

P057 Aaron Hardin, University of California, San Francisco

Polymorphisms in chromatin accessibility state within *D. melanogaster*

The early embryo of *D. melanogaster* has several of the best studied gene regulatory networks in animals. In particular, the factors that regulate anterior-posterior patterning were identified by genetic methods several decades ago, and we now well understand many of their activities, expression patterns and targets. Previous work in the Eisen lab has shown that these factors bind to thousands of regions across the genome. Our lab has measured transcription factor binding in both D. melanogaster and the relatively closely related species D. yakuba but have been unable to predict the underlying sequence changes that influence these changes due to the large number of polymorphisms between species. However, correlated changes in binding across factors suggests that the underlying chromatin state may be playing a significant role in binding divergence. We have now examined chromatin state in several natural isolates of D. melanogaster by measuring genome wide DNase-hypersensitivity with high-throughput sequencing during early embryogenesis. The regions of hypersensitivity strongly correlate with nucleosome free regions and regions of bound transcription factors. In order to identify the sequence polymorphisms and correlate these with changes in chromatin state, we have pooled DNase treated chromatin from pairs of natural isolates and compared the frequency of recovered regions to the frequency of the polymorphisms present in each sample. We identified several thousand polymorphism associated with differential DNase accessibility, often proximal or overlapping gene enhancers indicating that these polymorphisms could be contributing to expression variability.

P058 Laurel Hiebert, University of Oregon

Origin of a metamorphic life cycle in the phylum Nemertea: Hidden invaginated rudiments as possible precursors to imaginal discs

Within the marine invertebrate phylum Nemertea, one clade, Pilidiophora, exhibits maximally-indirect development via the pilidium larva, while other nemerteans have a more direct development. Uniquely among animals, the nemertean juvenile develops inside the pilidium from a set of invaginated rudiments, called imaginal discs, that grow and fuse around the larval gut. Pilidial development culminates in catastrophic metamorphosis. While the juvenile/adult body plan is conserved, the pilidium is a novel body plan that evolved within the phylum. In order to understand how this novel body plan arose we studied the expression of the Hox and other genes during development of the pilidiophoran Micrura alaskensis and the direct-developing Pantinonemertes californiensis (Hoplonemertea). We isolated nine Hox genes from M. alaskensis and found that their developmental expression is restricted to the trunk imaginal discs. At the same time we found that Six3/6 gene is expressed in the cephalic discs of M. alaskensis. Invaginated rudiments are also present in the direct-developing species, and are possibly homologous to pilidial imaginal discs. Cell proliferation patterns as well as expression of Hox and Six3/6 genes support the hypothesis of homology. We hypothesize that the tucked-away juvenile rudiments may be precursors to true imaginal discs as found in Pilidiophora. Interestingly, no Hox expression is ever observed in the pilidial body, suggesting that larva and juvenile are patterned differently. This may explain how larval and juvenile development became decoupled, resulting in a diversity of pilidial morphologies within the Pilidiophora.

P059 Shinnosuke Higuchi, RIKEN

Evolutionary developmental biology of cloacal muscles in the vertebrates

Vertebrates have evolved cloaca for excretion and reproduction. In amniotes, certain skeletal muscles are involved in constriction of cloaca. According to classical papers of comparative morphology, an array of skeletal muscles was distinguished from body wall muscles among the vertebrates including the cyclostomes. However, homology of these "cloacal muscles" has been elusive. To understand the evolutionary process of cloacal muscles, we compared cloacal muscle development and innervation patterns among cyclostomes (lamprey and hagfish), chondrichthyans (shark) and amniotes (chicken). We confirmed in cyclostomes, that striated muscles develop around the cloaca independently of the body wall muscles. In the shark, the muscles surrounding the cloaca are innervated by a branch of the spinal nerve that also innervates the pelvic fin. Although nerve plexus in the shark was not distinct as in amniotes, anastomoses formed between adjacent spinal nerves proximal to the branching of fin and cloacal nerves, suggesting the evolutionary continuity in nerve topography between elasmobranchs and amniotes. We observed that the cloacal muscle develop from a subpopulation of the muscle precursor cells of the pelvic

fin muscles in the shark, and it is reminiscent of the common developmental origin of the cloacal and hind limb muscles in the amniotes. However, positions of divergence of these two muscle subdivisions differed: the position of divergence is found within the body wall in the shark, but within the hind limb bud in the amniotes. It is likely that the cloacal muscle primordia had changed their migratory route laterally in the evolution towards the amniotes.

P060 Dorit Hockman, University of Oxford

The development and evolution of vertebrate oxygen-sensing cells

When oxygen levels in the blood or surrounding air/water drop below a set point (hypoxia), oxygen-sensing cells release neurotransmitters to stimulate afferent glossopharyngeal and/or vagal nerve endings, triggering increased ventilation via the respiratory reflex. In amniotes, these comprise carotid body glomus cells and 'pulmonary neuroendocrine cells' in the lung airway epithelium. In anamniotes, they comprise 'neuroepithelial cells' (NECs) in the gill and orobranchial epithelia (including the lung epithelium of air-breathing ray-finned fishes, lobe-finned lungfishes and amphibians), plus the carotid labyrinth of amphibians. It is currently assumed that carotid body glomus cells, which are neural crest-derived and develop in association with the third pharyngeal arch artery, evolved from gill NECs, which also develop in association with pharyngeal arch arteries. However, this has never been tested. Using vital dye labelling, neural fold grafts, genetic lineage-tracing and analysis of zebrafish mutants lacking all neural crest cells, we show that the serotonergic NECs in the gill and orobranchial epithelia of zebrafish, frog and lamprey are not neural crest-derived, hence cannot be homologous to carotid body glomus cells. Instead, NECs are most likely homologous to pulmonary neuroendocrine cells, which are endoderm-derived. In neonatal mammals, neural crest-derived adrenal chromaffin cells are sensitive to hypoxia, and at least some hypoxia-responsive chromaffin cells persist in the adult. We propose that carotid body glomus cells evolved from scattered chromaffin cells associated with the large blood vessels of the pharyngeal arches.

P061 Jason Hodin, Stanford University

Shaken and spurred: EcoEvoDevo of larval settlement in response to nearshore turbulence

Metamorphic life cycles –characterized by substantial morphological transformation between multicellular phases– have evolved repeatedly from non-metamorphic ancestral life cycles in animals and non-animals. In the ocean, metamorphosis in animals is often associated with irreversible settlement of larvae from the plankton to the seafloor. This dramatic transformation involves the integration of multiple environmental and internal signals by the developing larvae to control the proper timing and location of settlement, subsequently impacting the successful recruitment of larvae into benthic populations. Therefore, there is a fascinating interplay between ecology, evolution and development at metamorphosis that makes it an under-appreciated archetype for EcoEvoDevo studies. Here we explore one aspect of the

signal integration that occurs as planktonic larvae approach their juvenile habitat. In animals whose adults live on wave-impacted shores, their dispersing larvae must pass through waters of increasing turbulence intensity as they approach suitable settlement habitat. By contrast, dispersing larvae of species whose adults live on calmer shores or in deeper subtidal locations would not experience such increases in turbulence intensity before settlement. We show that the larvae of seashore-dwelling sea urchins are activated to settle precociously by such exposure to intense turbulence, indicating a trade-off between continued larval growth and the likelihood of encountering suitable juvenile habitat.

P062 Thorsten Horn, University of Cologne

From extra- to embryonic: The changing roles of Dorsocross during insect evolution

The T-box transcription factor Dorsocross (Doc) is highly pleiotropic in Drosophila development. It is necessary for patterning the dorsal-ventral axis in the hindgut, for specification of heart cells and wing primordia, and it has a morphogenetic function in folding of the wing disc. However, the most crucial function of Doc is the maintenance of the extraembryonic membrane (EEM). Drosophila only possesses a single EEM, the amnioserosa, which is evolutionarily derived and represents a secondary reduction. Here we present our analysis of Doc's functions in the beetle Tribolium, which is more representative for insect extraembryonic development, as it has a full set of EEMs, the amnion and the serosa. As in most insects, they are highly dynamic and actively enclose and later withdraw from the embryo. RNAi against Doc in Tribolium revealed three defects at different developmental stages, all linked to EEM morphogenetic movements and tissue topography. However, in contrast to Drosophila, we did not find any embryonic defect in the Doc knockdowns. We then compared the expression of Doc and some of its potential upstream regulators and downstream target genes between Drosophila and Tribolium. We found more relaxed gene regulatory networks, with some interactions (e.g. dpp-Doc) suggesting feedback loops rather than the hierarchical regulation typical of Drosophila, even when the same molecular players are involved. Combined with preliminary results from the bug Oncopeltus, our findings point towards an ancestral role of Doc in morphogenesis rather than tissue specification or maintenance. Furthermore, Doc's function in Tribolium seems to be predominantly extraembryonic.

P063 Yi-Min Hsiao, National Taiwan University

Embryonic development of the parthenogenetic and viviparous pea aphid: Axis determination and pattern formation

The pea aphid *Acyrthosiphon pisum*, a rising model for genomics and developmental studies, propagate offspring parthenogenetically and viviparously. Hence oogenesis is followed by embryogenesis within the same ovariole. Previously, we identified anterior localization of *hunchback* (*Aphb*) mRNA in oocytes and early embryos of the asexual pea aphid, suggesting that the symmetry breaking in the anterior pole specifies the anterior axis. Expression of *caudal* (*Apcad*) mRNA,

however, was first identified in the egg posterior until blastoderm formation, implicating that determination of the anteroposterior (AP) axis is not synchronous in the asexual pea aphid. For studying the formation of the dorsoventral (DV) axis, we detected expressions of *decapentaplegic* (*Apdpp*) and *short gastrulation* (*Apsog*), both of which are toolkit genes for DV axis formation in insects. Asymmetric distribution of mRNAs transcribed by the four *Apdpp* genes (*Apdpp1-4*) was not observed whilst *Apsog* could be identified in the ventral region of the cellularized embryos. Before blastoderm formation, mRNAs of both genes were randomly distributed. We thus argue whether DV axis is determined after the embryo becomes cellularized. Interestingly, we identified colocalization of mRNA transcribed by *Aphb* and the germline gene *Apvas1* in the germ cells throughout development. Whether *Aphb* is involved in the germline development requires further investigation using functional tools, such as RNA interference. Besides, we also examined the patterning of *Hox* genes. All of the efforts described above, we expect, can enable us to understand how a parthenogenetic and viviparous aphid constructs its body plan at the molecular basis.

P064 Keita Ikegami, Okinawa Institute of Science and Technology
Towards understanding of the phylotypic stage in the ascidian *Ciona intestinalis*

In the vertebrate embryogenesis, developmental periods where embryos morphologically resembles the most are called phylotypic stage. The phylotypic stage also believed to characterizes the trait of the animal in the phylum (or sub-phylum) level. Recent advent of high throughput instruments enabled to measure comprehensive gene expressions of the whole-embryos which led to suggest phylotypic stages conserved in various animal phylum: germ band stage in the Arthropoda (2010), pharyngula stage in the Vertebrata (2011), and ventral closure stage in the Nematoda (2012). In this work, we conducted transcriptome analysis on the Urochordata to approximate the phylotypic stage using the ascidian Ciona intestinalis. From our analysis, late tail bud to larva stage were predicted as phylotypic stage in C. intestinalis.

P065 Paula Irles, Pontificia Universidad Catolica de Chile
Dynamics of follicular cell proliferation during insect oogenesis: From panoistic to meroistic ovaries

The proper ovarian follicle development is a main requirement for reproductive success of insect species. In the panoistic ovary, which is present in more basal insect, the oocyte is the responsible to provide all the materials needed for their own growth and is the source of mRNAs and proteins necessaries for embryo development. In the meroistic ovary type this action is performed by the nurse cells, a second germ line cell. The follicular epithelium which has a somatic cell origin envelops the oocyte during its maturation, exerting different critical functions according to the ovary type. Our research has been focused on different signaling pathways affecting the dynamics of the follicular epithelium, regulating proliferation, differentiation and cell death during oogenesis. The Hippo pathway has an important role in the regulation of

cell proliferation and cell death processes, finally determining the organ size. It exerts a crucial role in follicular cell proliferation in the panoistic ovary of the cockroach Blattella germanica. In addition, the interplay between the Hippo and Notch pathways in both ovary types evidenced clear differences in the transition from mitosis to endocycle program during vitellogenesis. At present, we are studying the dynamics of follicular epithelium in the earwigs Euborellia annulipes and Forficula auricularia (Dermaptera), believing that this insect order which has a polytrophic meroistic ovary but with a only big nurse cell, will bring strategic information to unveil how was done the transition from the panoistic to the meroistic ovary type.

P066 Johannes Jaeger, Centre for Genomic Regulation (CRG)

Life's Attractors: The Evolutionary and Developmental Dynamics of the Gap Gene System

We have carried out a comprehensive integrative analysis of the patterning capacity and the evolutionary potential of an experimentally tractable developmental gene network, the gap gene system involved in patterning and segment determination during early embryogenesis in dipteran insects. Using a reverse-engineering approach we have created data-driven mathematical models of the gap gene network in three species of flies: Drosophila melanogaster, the scuttle fly Megaselia abdita, and the moth midge Clogmia albipunctata. Comparative analysis of these models reveals evolution by system drift, which allows the network to compensate for differing maternal inputs in different species. Our work provides precise causal-mechanistic mechanisms for this sort of compensatory evolution, and shows how random changes in regulatory interactions lead to non-random changes of gene expression dynamics. In addition, our models reveal a novel mechanism for the dynamic positioning of gap domains in different species. These shifting domains are driven by a damped oscillator which causes nuclei to express a stereotypical succession of gap genes. This mechanism shows surprising structural similarities to the short germband mode of segmentation, implying that long- and shortgerm band embryos are patterned by developmental processes that are much more similar to each other than previously thought.

P067 Alys Cheatle Jarvela, University of Maryland

A gene regulatory network for sea star apical organ development links neurogenesis to AP patterning

Studying the genetic mechanisms underlying neurogenesis in a variety of organisms will improve our understanding of the origins of the central nervous system (CNS). Although nervous systems are extraordinarily diverse across animal phyla, many disparate taxa have a free swimming larva, which forms an anterior concentration of neurons, or apical organ. The number of neurons in these organs is highly variable, even among members of the same clade. Almost nothing is known about how neurogenesis is controlled during the formation of these apical organ neurons in any taxa. Using gene perturbation and expression techniques, we find that neurogenesis in the sea star larvae begins with soxc-expressing multipotent progenitors. These give rise to committed

progenitors, which express lhx2/9. We see that both soxc and lhx2/9-expressing cells are capable of undergoing both asymmetric divisions, which allow for progression towards a particular neural fate, and also symmetric proliferative divisions. Nested concentric domains of gene expression along the anterior-posterior (AP) axis, which have been observed in a variety of metazoans, control neurogenesis by promoting either proliferation or differentiation of neural progenitors. Experimental disruptions to these domains affect neuronal output. This work explains how spatial patterning in the ectoderm controls progression of neurogenesis. Importantly, evolutionary changes to the expression domains of AP neuronal patterning genes result in changes in proliferation zone size. Such changes in AP patterning may lead to differences in apical organ size seen among echinoderm larvae and also provide a mechanism for understanding the great evolutionary expansions of CNS in vertebrates.

P068 Kathryn Kavanagh, University of Massachusetts
Shared Rules of Development Predict Patterns of Evolution in Vertebrate Segmentation
We have found that non-homologous structures in the body follow remarkably similar design rules. Despite the different genetic and developmental origins, segmented structures of the skeleton like limbs, digits, molars, somites, and vertebrae vary and evolve different size proportions in highly predictable ways. The variations can be modeled by an activator-inhibitor model, the inhibitory cascade. Model outcomes match extremely well with embryo experimental outcomes and also with selected lines, and microevolutionary and macroevolutionary patterns of variation in a large, diverse sample of tetrapods. We find that all these segmented systems follow the expectations of the model, which include a middle segment of one-third of the total size, a proximo-distal trade-off in size, and variance apportioned parabolically. This deeply convergent segmentation rule raises questions of the extent of developmental bias in essential structural design of animal bodies. These developmental signals persist into adult forms, however additional modifications at later stages or integrating other elements can produce variations outside of this developmentally constrained morphospace. The result suggests that skeletons may use self-organizing principles that are independent of specific genetic underpinnings.

P069 Nathan Kenny, The Chinese University of Hong Kong
New techniques and protocols in a novel model arthropod, the cherry shrimp *Neocaridina denticulate*
Arthropods are the most speciose known phylum, and for the past century they, and particularly insects, have played a leading role in evolutionary developmental research. The dominance of insect-based work has only increased with the advent of genomic sequence data, and has risked skewing our perception of the drivers of evolutionary and phenotypic change. Whether findings originating in the Insecta are truly representative of arthropods, let alone metazoan life as a whole, is proving increasingly contentious. Novel model organisms have a key role in testing these hypotheses, with better outgroups allowing more

robust conclusions to be drawn as to the universality of results across animal phylogeny. One candidate organism for such a role as an outgroup to the Insecta, and as a useful model organism in its own right, is the cherry shrimp Neocaridina denticulata. This species is hardy, grown commercially for human consumption and is available worldwide courtesy of the pet trade. Genomic resources for this organism have been published, with efforts continuing on the establishment of transcriptomic resources. Here we describe our work on the manipulation of embryos and the imaging of live and fixed samples of this species, proving it amenable to a range of common techniques used in developmental biology. This has allowed us to explore the evolution of a number of arthropod synapomorphies in the Decapoda. The experimental tractability of this shrimp will allow such work to continue in earnest, and recommends it as a model to any field that wishes to explore arthropod evolution from the perspective of the Crustacea.

P070 Pierre Kerner, CNRS

Evolution of Prdm genes in animals: insights from comparative genomics and gene expression studies

Prdm genes encode transcription factors with a PRDI-BF1 and RIZ homology (PR) domain and a variable number of zinc finger motifs. These genes show a wide variety of functions. In particular, several Prdm genes, such as Prdm1 and Prdm14, have important roles in somatic pluripotent stem cells and in primordial germ cells. Other genes, such as Prdm8, Prdm12, and Prdm13, are expressed in specific neural populations and are required for the proper development of these neural cells. Whereas the functions of Prdm genes have been carefully studied in some vertebrates little is known about the evolution of this gene family. We have searched for Prdm genes in the fully sequenced genomes of 91 different animal species representative of all the main animal lineages. We identified a total number of more than 900 Prdm genes in these species, the number of Prdm genes per species ranging from 2 to 19 depending on the species. To better understand how the Prdm gene family has evolved in metazoans, we performed phylogenetic analyses using the large set of Prdm genes we have identified. These analyses allowed to define 14 different subfamilies of Prdm genes and to establish that 11 of them are ancestral to bilaterian animals. Detailed analysis allowed to define the gene duplication and gene loss events that occurred in the different animal lineages. By studying a large number of non-animal genomes, we also defined the most likely evolutionary origin of this gene family. To get insight into the evolution of the functions of these genes in bilaterian animals, we cloned the full set of Prdm genesfrom the emerging model species, the annelid Platynereis dumerilii, a slow-evolving species that is distantly related to both vertebrates and arthropods. Expression patterns of the cloned genes will be reported. Together, our data provide new insights in the evolution of this important family of transcription factors.

P071 Ryan Kerney, Gettysburg College

How does algae enter embryonic tissues and cells of the spotted salamander (*Ambystoma maculatum*); a dual RNA-Seq Approach?

The symbiosis between the eukaryotic green alga Oophila amblystomatis and embryos of the spotted salamander, Ambystoma maculatum, is unique among vertebrates. Early exclusion experiments determined the association to be a mutualism, with measureable benefits to both the symbiont and host. Unlike other vertebrate-microbial mutualisms, this symbiosis also includes algal symbionts entering host tissues and cells. Several aspects of both host and symbiont biology, along with substantial research on closely related species to both partners, makes this association uniquely poised to determine the mechanisms of algal cell entry. This study describes a dual-RNASeq approach to determining the transcriptional changes to both symbiont and host that are associated with the process of cellular entry. Near single-cell resolution of these transcriptional changes reveal parallels between algal cell entry and the intracellular invasion of pathogens. Up regulated host genes are associated with an innate immune response and modifications to the extracellular matrix. These results contrast pathogenic cellular invasion processes, such as the invasion of Batracochytrium dendrobatidis into amphibian epithelial cells. Other transcriptional responses, and preliminary in vivo co-culturing results, are consistent with the host actively recruiting algal cell entry. We will present these novel data and discuss their implications for this unique vertebrate-algal association.

P072 Teiya Kijimoto, West Virginia University Davis College

Interaction between organisms and the environment: developmental regulation of polyphenism in horned beetle

Organisms are constantly exposed to environmental changes and adapt to those changes by adjusting morphology and/or physiology. In animals in particular, environmental changes are commonly integrated into developmental processes underlying traits critical for migration and/or reproduction. In many species such adjustments result in the facultative development of two or more alternative morphs, a phenomenon referred to as polyphenism. Insects are especially famous for the abundant and elaborate polyphenism. I would like to discuss the molecular basis of the developmental regulation of polyphenism in general, and that of sexually selected alternative male morphologies in horned beetles in particular. I will first show that genes and pathways critical for the developmental regulation of insect appendages such as legs or genitalia are also critical for the development of beetle horns. I will then propose a possible evolutionary scenario how horned beetle polyphenisms may have originated and diversified.

P073 Evan Kingsley, Harvard University

Developmental mechanisms responsible for tail length variation in deer mice

Variation in the shape, size, and number of segments along the vertebral column underlies a vast amount of vertebrate diversity. Although the molecular pathways controlling vertebrate segmentation

during normal development are well understood, the genetic and developmental underpinnings responsible for the tremendous variation in size and number of vertebrae are relatively unexplored. To study the mechanisms that generate this variation, we investigated differences in tail length among populations of the deer mouse, Peromyscus maniculatus, a tractable system in which to better understand how natural selection, acting through changes in embryogenesis, shapes morphological variation. Previous work shows that these mice use their tails extensively while climbing. Using a phylogeographic framework, we show that longer tails have evolved independently in different populations of forest-dwelling mice. Closer investigation of the underlying morphology shows that long-tailed mice have both (1) a greater number of tail vertebrae and (2) individually longer vertebrae, compared to ancestral short-tailed mice. We used quantitative trait locus mapping to uncover six loci that influence differences in total tail length (3 associated with vertebral length and 3 with vertebrae number). When combined with comparative data from in situ hybridization and quantitative measurements of tissue dynamics during somitogenesis, we find that embryos of forest mice make more segments because they produce more presomitic mesoderm. Together, these results pinpoint the ways that natural selection modifies development to produce the repeated evolution of an evolutionarily important trait and suggest that there are a limited number of ways that long tails can evolve.

P074 Jamie Kostyun, Indiana University

Evolutionary Developmental Genetics of Floral Diversity in Jaltomata (Solanaceae)

One goal of evolutionary development studies is to understand the genetic and developmental changes underlying the evolution of phenotypic novelty and subsequent diversification, a key process contributing to biodiversity. I focus on floral diversity, by examining floral trait differences among 12 closely related species within the genus Jaltomata (Solanaceae). Flowers of these species span multiple axes of variation, including petal and nectar color, overall size, and aspects of morphology – providing an excellent opportunity to dissect numerous traits contributing to floral disparity. My goal is to identify genetic and developmental changes controlling these observed floral trait differences, so as to better understand floral evolution in this highly diverse system.

P075 Rie Kusakabe, RIKEN

Development and Evolution of Mesodermal Components of the Lamprey

The lamprey, one of the only two extant cyclostomes, is a key animal in developmental biology of early vertebrates. Lampreys retain a variety of ancestral features of the vertebrates, including absence of jaws, paired fins and epaxial/hypaxial distinction of the trunk skeletal musculature. We have studied the developmental mechanisms underlying the myogenesis of the Japanese lamprey, Lethenteron japonicum, and have discovered that the hypobranchial muscle of the lamprey undergoes a similar developmental process to that found in the tongue muscles of

jawed vertebrates. Precursors of the hypobranchial muscle emerge from the ventral edges of the somites as streams of undifferentiated myoblasts. These myoblasts migrate anteriorly and line up in two bilateral rows at the ventral floor of the pharynx where they undergo terminal differentiation. Alongside of the migratory pathway are the pharyngeal arches, the muscles of which are derived from the unsegmented head mesoderm, and the heart primordium, which develops posterior to the pharynx. We aim to reveal the cellular and molecular characteristics of each mesodermal component of the so-called circumpharyngeal region of the lamprey and illustrate how they relate to the complexity and diversification of the vertebrate morphology.

P076 Kenro Kusumi, Arizona State University

The Evolution of Regenerative and Developmental Gene Networks in Vertebrates: Insights from Comparative Genomic Studies

Many vertebrates display the ability to regenerate appendages and complex tissues such as spinal cord, but classic models such as the mouse and chick have been unsuitable for regenerative studies since mammals and birds have very limited capacity. With the availability of whole genome sequences and functional genetic technologies for reptilian, amphibian, and teleost models, comparative studies of regeneration are now possible. Lizards, which are amniote vertebrates like humans, are able to lose and regenerate a functional tail with regrowth and patterning of spinal cord, cartilage, muscle, vasculature, and skin. Building on our annotation of the green anole genome, we analyzed the mRNA and microRNA transcriptomes during tail regeneration in the green anole lizard, Anolis carolinensis. Transcriptomic analysis revealed 326 differentially expressed genes, of which 302 have clear human orthologues, regulating wound and immune response, hormonal regulation, and musculoskeletal development. MicroRNA sequencing of lizard regenerating tail and associated tissues revealed both novel and known microRNA precursor families. By using a comparative systems biology approach, we are working to discover conserved gene regulatory networks for regeneration in vertebrates. Comparing our lizard transcriptomic data with manually curated, public RNA-seq and microarray data sets from anamniote models, including the salamander, Xenopus frog, and zebrafish, we have already identified common patterns of activation of the canonical Wnt and Wnt5-calcium signaling pathways. By combining evo-devo and genomic approaches, we are working to identify conserved and convergent gene regulatory networks that may impact on future regenerative medical therapies.

P077 Chun Wai Kwan, University of Chicago

A Self-Regulatory BMP Signaling Circuit Drives Sequential Serosa and Amnion Specification In the Scuttle Fly Megaselia abdita

A central question in evolutionary developmental biology is how morphological evolution relates to changes in genetic and developmental processes. Self-regulatory signaling mechanisms have been shown to be important in numerous developmental processes but how their evolution affects cell fate specification and morphological

change remains poorly understood. We found that self-regulatory BMP signaling accounts for serosa and amnion specification in flies and has been altered in the Drosophila lineage, where these tissues have merged into one, called amnioserosa. Amnioserosa specification in Drosophila requires refinement of BMP signaling from a shallow gradient into a sharp peak. This refinement is achieved during dorso-ventral pattering by means of a self-regulatory feedback loop. Mathematical modeling of this process revealed that under certain parameter conditions, peak splitting of BMP signaling occurs. While peak splitting was not observed in Drosophila, we found that this modeling result closely matches the dynamic pattern of BMP activity in the scuttle fly Megaselia abdita. Megaselia is one of the closest relatives of Drosophila with distinct serosa and amnion tissues. Time-controlled knockdown of BMP signaling in this species suggests that peak spitting (or broadening) is critical for amnion specification. Our ongoing investigation indicates that Megaselia uses a self-regulatory BMP-signaling circuit, similar but not identical to that found in Drosophila, to sequentially drive serosa and amnion specification. Our work suggests that subtle changes in self-regulatory signaling mechanisms can dramatically alter cell fate specification and the path of morphological evolution.

P078 Alexis Lainoff, University of California, San Francisco

Comparative examination of early odontogenic gene expression in amniotes

A well-known tenet of murine tooth development is that BMP4 and FGF8 antagonistically initiate odontogenesis, but whether this tenet is conserved across amniotes is largely unexplored. Moreover, changes in BMP4-signaling have previously been implicated in evolutionary tooth loss in Aves. Here we demonstrate that Bmp4, Msx1, and Msx2 expression is limited proximally in the red-eared slider turtle (Trachemys scripta) mandible at stages equivalent to those at which odontogenesis is initiated in mice, a similar finding to previously reported results in chicks. To address whether the limited domains in the turtle and the chicken indicate an evolutionary molecular parallelism, or whether the domains simply constitute an ancestral phenotype, we assessed gene expression in a toothed reptile (the American alligator, Alligator mississippiensis) and a toothed non-placental mammal (the gray short-tailed opossum, Monodelphis domestica). We demonstrate that the Bmp4 domain is limited proximally in M. domestica and that the Fgf8 domain is limited distally in A. mississippiensis just preceding odontogenesis. Additionally, we show that Msx1 and Msx2 expression patterns in these species differ from those found in mice. Our data suggest that a limited Bmp4 domain does not necessarily correlate with edentulism, and reveal that the initiation of odontogenesis in non-murine amniotes is more complex than previously imagined. Our data also suggest a partially conserved odontogenic program in T. scripta, as indicated by conserved Pitx2, Pax9, and Barx1 expression patterns and by the presence of a Shh-expressing palatal epithelium, which we hypothesize may represent potential dental rudiments based on the Testudinata fossil record

P079 Mara Laslo, Harvard University

Developmental expression of *TRα* and *TRβ* in the limb of the direct-developing frog *Eleutherodactylus coqui*

Direct development has evolved independently in at least a dozen Anuran lineages. Direct-developing frogs, including the Puerto Rican coquí, *Eleutherodactylus coqui*, hatch from terrestrial eggs as miniature adults. Their embryonic development is characterized by precocious formation of adult features, such as limbs. In metamorphosing frogs, limb development is mediated by thyroid hormone (TH) during metamorphosis. Changes in TH signaling could underlie the evolution of direct development. Specifically, changes in temporal or spatial expression of the nuclear thyroid receptor α (TRα) or thyroid receptor β (TRβ) in target tissue could facilitate the early development of limbs. qRT-PCR was used to examine *TRα* and *TRβ* expression in the developing *E. coqui* limb. *TRα* expression is significantly higher than *TRβ* expression throughout most of limb development and peaks at stage 10. *TRβ* expression is low during early development and rises significantly at stage 10, when thyroid follicles appear. Prior studies suggest limb development in direct developing frogs is independent of TH. However, these patterns share some similarities to *TRα* and *TRβ* expression patterns observed in the developing limb of the metamorphosing frog *Xenopus laevis*, where limb development is dependent on TH. These data, together with a TH developmental profile, suggest that the *E. coqui* limb is potentially TH competent and thyroid mediated development may begin earlier than previously thought. Describing the mechanism of direct development is an important first step and will serve as a comparison to examine the developmental basis of this life history strategy in other amphibian groups.

P080 Michael Layden, Lehigh University

Bidirectional Notch-Delta signaling is a highly conserved regulator of neural differentiation in animals

Notch-Delta signaling is a metazoan innovation. Extensive investigations in bilaterians suggest that one ancestral function of this pathway is to regulate cellular differentiation. We investigated notch and delta homologs in the cnidarian Nematostella vectensis. Notch represses neuronal differentiation by suppressing expression of the neurogenic gene NvashA (Layden and Martindale, EvoDevo 2014). We recently investigated the ancestral role of Delta in Notch-Delta signaling. Synthetic activation of Nvdelta by misexpressing its intracellular domain (NvdeltaICD) induces NvashA expression. Moreover, NvdeltaICD misexpression decreases cell number at later stages of development in part by suppressing cell proliferation, a hallmark of cellular differentiation. The activated NvDelta intracellular domain localizes to the nucleus suggesting that the observed phenotypes are downstream of transcriptional regulation, and we are currently confirming this hypothesis. Our current model is that Notch-Delta activity between two cells results in Notch activation in one cell suppressing differentiation by actively inhibiting differentiation markers and promoting cell proliferation.

Conversely, Delta activity in the neighboring cell suppresses the cell cycle and promotes expression of differentiation markers. Taken together our data suggest that bidirectional Notch-Delta signaling in Nematostella regulates neuronal differentiation, suggest that Notch-Delta regulation of differentiation may be the ancestral function of this pathway, and argue that bidirectional Notch-Delta signaling is deeply conserved in animals. We are currently exploiting the unique biology of the Nematostella embryo to identify targets of activated NvDelta in hopes of better understanding the mechanisms of bidirectional Notch-Delta signaling in all animals.

P081 Cris Ledon-Rettig, Indiana University

The condition-dependent transcriptome of the sexually dimorphic beetle *Onthophagus taurus*: Contributions of, and interactions between, sex, nutrition, and body region

Some of the most spectacular examples of intraspecific diversity are generated via sexual dimorphisms: Although males and females typically possess nearly identical genomes, they often exhibit drastic phenotypic differences, from morphology to behavior, and physiology to disease risk. However, not all traits are equally sexually dimorphic; instead, individuals are mosaics of tissues that vary in their ability to exhibit dimorphism. Furthermore, a trait's degree of sexual dimorphism is frequently modified by environmental conditions, in particular nutrition. Thus, to understand why and how sexual dimorphisms evolve the way they do, we need to understand the developmental genetic mechanisms that enable variation in sexual dimorphism across tissues and nutritional conditions. However, our understanding of these mechanisms remains poor. Here, we investigate the transcriptomic basis of sexual dimorphism in the bull-headed dung beetle Onthophagus taurus. In this species, males and females differ substantially in tissue-specific growth responses to nutritional variation, generating remarkable and complex sexual- and within-male dimorphisms. By comparing genome-wide expression associated with nutrition-dependent sexual dimorphism across four tissues and two nutritional conditions, we examine basic hypotheses regarding the developmental-genetic mechanisms underlying, and evolutionary consequences of sexually dimorphic and nutritionally sensitive phenotypes. Specifically, we investigate (i) the shared developmental-genetic underpinnings of nutrition-dependence and sexual dimorphism, (ii) correlations between sex-biased gene expression and sexual dimorphism in morphology, (iii) the resolution of sexual conflict via tissue-specific, sex-biased gene expression, (iv) the functional outcomes of sex-biased gene expression, and (v) the accumulation of genetic variation in sex-biased genes.

P082 Ezra Lencer, Cornell University

Evolution and Development of Pupfish Skull Morphology

Understanding the origins of novel phenotypic variation is fundamental to the study of biological diversity. Many studies have focused on the role of selection in driving phenotypic change; however, equally important is how phenotypic variation is generated. We investigate the genetic and developmental sources of skull modifications in a

geologically recent radiation of three sympatric pupfish species (genus Cyprinodon) endemic to the lakes of San Salvador Island, Bahamas. These pupfish differ dramatically in jaw morphology exhibiting elongate upturned lower jaws used to remove scales from other fish, and robust squat jaws nested under nasal and maxillary extensions associated with the transition to a shelled prey diet. We show how differences in skull morphology emerge over development as a consequence of differential growth of oral jaw elements. Lengthening or shortening of these elements affects the shape of jaw bones as well as the relative placement of bones in the skull leading to derived morphologies unlike those exhibited by any of the ~50 other species in the genus. Whole mount in situ hybridization results to date indicate that the spatial and temporal regulation of genes known to affect skull morphology in other taxa are not differentially expressed among pupfish species. We furthermore take an RNA-seq approach to measure regulatory divergence in developing skull tissue among the three San Salvador species at multiple developmental stages that span embryonic development and larval growth. These data will identify novel sources of skull variation, an ecologically important trait that varies dramatically across vertebrates.

P083 Maryna Lesoway, McGill University

Ecology, evolution, and development of nutritive embryos in the calyptraeid gastropods

Polyphenisms are discrete phenotypic responses to varied environmental stimuli. Nutritive embryos, embryos that arrest development and are ingested by their normally developing siblings, may represent a developmental polyphenism. Although they are found in diverse groups including frogs, ants, and marine snails, little is known about their development and evolution. Combining ecology, embryology, gene expression, and development, we studied nutritive embryos in the calyptraeid gastropods. We focused on two species, Crepidula navicella and Calyptraea lichen. Both are able to produce nutritive embryos, but C. lichen can switch developmental mode, producing either mixed broods that include nutritive embryos or broods of viable embryos that hatch as free-swimming larvae. Analysis of brood composition during seasonal changes in upwelling suggests that C. lichen increases production of mixed broods during upwelling, while C. navicella does not adjust embryo allocation. Nutritive embryos of C. navicella are morphologically indistinguishable from viable embryos until shortly after gastrulation. Early distinctions between viable and nutritive embryos may be influenced by precocious activation of MAPK (ERK 1/2). Early MAPK activation correlates with apoptotic signals in some cleavage-stage embryos of C. navicella, and a similar pattern is found in C. lichen. High throughput sequencing of transcriptional differences between viable and gastrula-like nutritive embryos of C. navicella shows that viable embryos express transcripts associated with tissue development, while nutritive embryos express transcripts that control apoptosis and cellular events. These data highlight the importance of combining multiple levels of biological organization to understand the evolution of development.

P084 James Lewis, Cornell University

Epigenetic profiling of functional loci driving wing pattern variation in phenotypically diverse races of *Heliconius erato*

With over twenty described co-radiating wing pattern morphs, Heliconius erato and Heliconius melpomene butterflies have become an important model for understanding the evolutionary and mechanistic basis of morphological change. Prior mapping and expression studies have identified only three genomic loci controlling the majority of wing pattern variation within and between H. erato and H. melpomene races. Recent studies in model organisms have demonstrated the power of epigenetic profiling for identifying regulatory elements controlling gene expression. Here we introduce epigenetic profiling of regulatory elements as a tool for precisely characterizing the functional regulatory loci driving population divergence associated with wing pattern evolution in H. erato. We use whole genome assays of chromatin accessibility to mark active regulatory loci, and chromatin immunoprecipitation and sequencing (ChIP-seq) for H3K4me3 and H3K27ac histone variants to characterize promoter and enhancer activity. We present evidence of strong conservation of functional elements in the Heliconius genome despite significant nucleotide variation and assortative mating between populations. Combining functional assays of epigenomic signals with published population genomic signatures of selection, we show current evidence for specific regulatory loci controlling red color pattern variation in H. erato races. We will also briefly address the challenges of adopting functional genomics to non-model organisms, and the potential for assaying developmental networks with ChIP-seq using antibodies to transcription factor proteins.

P085 Bishuang Li, University of Rochester

Characterization of the Locus Controlling Wing Dimorphism in Male Pea Aphids

Morphological diversity is widespread among organisms, allowing them to adapt to various environments. The winged and wingless morphs displayed by male pea aphids provide an excellent system to study the genetic mechanisms that generate phenotypic diversity. Our previous mapping results have shown that a single locus (~130kb) on the X chromosome, called aphicarus (api), controls the suite of phenotypic traits associated with the different morphs. To identify the api gene, we performed an association study. Using Illumina sequencing of natural populations in France and USA, we found extremely high sequence divergence (1.3% in exons, higher outside of exons) between winged and wingless males within a large region (~60kb containing nine genes) at api. Many SNPs were perfectly associated with the male phenotypes. This result suggests there is a low recombination rate in this region, and that the alternative male phenotypes are maintained via an inversion and/or balancing selection. This result also supports the hypothesis that the wing dimorphism in male pea aphids has a single, possibly ancient origin. To narrow down an api candidate gene, we performed qRT-PCR of the nine genes in the api region on first instar and adult individuals. Two genes showed significant expression level differences between

morphs at both stages, suggesting that these are good api candidates and that a regulatory change may cause the phenotypic differences. Overall, our results identify promising candidates for further study and will ultimately provide significant insight into the molecular basis of morphological diversity.

P086 Irene Liao, Duke University

Analysis of candidate genes involved in nectar loss in the evolution of the selfing syndrome

In flowering plants, the shift from outcrossing to self-fertilization is one of the most common evolutionary transitions. This transition is typically associated with changes in several morphological characters, collectively known as the selfing syndrome. While one study has shown that natural selection drives reduced flower size in selfing plants, evidence for other syndrome traits is lacking. I am examining this issue by determining whether loss of nectar production is the result of selection or drift in the selfing morning glory Ipomoea lacunosa (Convolvulaceae). This species has a 95% selfing rate and exhibits typical selfing-syndrome characters (reduced floral size, pigment loss, reduced pollen production, and no nectar production) compared to its outcrossing sister species I. cordatotriloba. As a first step in this analysis, I have identified seven candidate genes underlying nectar loss, five of which are involved in synthesizing jasmonic acid. Using the draft genomes of both Ipomoea species, I found no differences in amino-acid sequences in the candidate gene homologs between the two species. Therefore, this project proposes to assess RNA expression differences in these homologs between these two species and test the co-segregation of molecular markers and nectar volume in an F2 mapping population. These two experiments will identify whether these candidates are involved in the loss of nectar production in I. lacunosa. This will lay the groundwork for molecular and field experiments for examining the evolutionary forces driving nectar loss in the evolution of the selfing syndrome.

P087 Rong-Chien Lin, Duke University

Genetic basis of an anther color polymorphism in *Erythronium umbilicatum* (Liliaceae)

How a polymorphism is maintained in natural populations has long interested evolutionary biologists. Accumulating evidence indicates that the genetic or phenotypic variation is maintained by some form of balancing selection, rather than reflecting accumulation of neutral mutations or a transient phrase where particular variants are replacing others. Floral color is one of the characters that frequently exhibit within-species variation, which often involves balancing selection mediated by pollinators. In an early spring herb Erythronium umbilicatum (Liliaceae), polymorphism is present in anther color. Each reproductive individual of E. umbilicatum produces a single, yellow flower. While the majority of the flowers bears purple anthers, the coexisting yellow-anthered flowers are not rare. As an self-incompatible herb with an ephemeral blooming period, E. umbilicatum may experience strong intra-specific competition for pollinators. Thus, E. umbilicatum provides an ideal system to study

the mechanism responsible for the anther color polymorphism. To understand whether such a polymorphism is maintained by balancing selection, our first goal is to identify the genetic changes associated with the anther color difference. We examined four genes (CHS, DFR, ANS, UFGT) in the anthocyanin biosynthetic pathway. Preliminary results show that the expression levels of these four genes are reduced in yellow anthers, implying that a transcription factor may be involved in downregulation for yellow pigmentation. Our next steps are to determine the identity of that transcription factor and to search for a signature of balancing selection on this gene.

P088 David Linz, Miami University

Exploring the molecular basis of insect wing evolution: A transcriptomic approach

We are studying the gene regulatory network of wing development in Tribolium (the flour beetle) and comparing it to that of the fly, Drosophila, to understand the molecular basis of morphological evolution. The wings of these two insects have become vastly different over evolutionary time. The fly has flight wings on T2, but has intensively modified wings (halteres) on T3. In contrast, the beetle has a pair of hardened protective structures (elytra) on T2, and uses the T3 hindwings for flight. We have been analyzing the function of potential "wing genes" (selected from previous Drosophila studies) in Tribolium wing development: a candidate gene approach. However, as these studies have progressed, the choices of candidate genes have become limited and created a fly-biased view of insect wing evolution. To gain a less biased view of insect wing evolution, we have started exploring genes that could be uniquely important for the beetle wing development. We are currently analyzing the Tribolium T2 and T3 wing transcriptomes, and comparing them to those of other insects including the Japanese stag beetle, Dorcus hopei, which will allow us to identify wing genes uniquely co-opted in the beetle lineage. We are also functionally assessing a subset of genes uniquely expressed in Tribolium wings by performing large scale RNAi in Tribolium. This work will provide the first comprehensive examination of genes present in the beetle wings and will provide further insights into the molecular mechanisms driving the evolution of morphology.

P089 David Luecke, University of California, Davis

Evolution of limb size results from tissue-specific, temporally restricted changes in cell proliferation rate

Change to the relative size of tissues, organs, or other biological compartments is a major generator of phenotypic diversity. How the complex suite of developmental and genetic growth controls evolves to produce this diversity is largely unknown. To address this question I investigate a recent transition in leg size found in the genus Drosophila. Males of the species Drosophila prolongata exhibit a drastic increase in the size of the first leg pair. These legs are used in novel combat and courtship behaviors, and their rapid evolution is likely driven by sexual selection. I initially investigated the developmental basis of this evolutionary transition. This work shows the increase in male first leg

size is established by the beginning of metamorphosis, at which point the first leg precursors are larger and have more cells than those of the second legs – a pattern not seen in females or in either sex of closely related species. I also show that the size difference emerges gradually during the final larval instar, and is initiated soon after the final larval molt by a brief increase in the cell proliferation rate of the first leg discs. I am currently investigating this window of dimorphic development to determine how cell proliferation responds to the developmental identifiers of sex and segment. Insights from this system will also produce candidates for other evolutionary diversifications facilitated by the developmentally constrained modification of cell proliferation in homologous tissues.

P090 Kate MacCord, Arizona State University
From Dental Evolution in the 19th Century to Developmental Evolution in the 21st
The question of how to explain the development and evolution of form is central to the modern field of Developmental Evolution. One way in which this question has been addressed is by bringing together multiple lines of evidence, such as paleontological and developmental data. A particularly good model for this is the mammalian dentition, which has an incredible fossil record and range of phenotypic diversity. Particularly successful in this venture is the lab of Jukka Jernvall at the University of Helsinki, where developmental biology has been merged with computational methods and paleontological data. While Jernvall's work is notably successful in addressing the origins of variation, questions remain about how to bring together and weigh multiple lines of evidence and what is necessary to account for both development and evolution. These questions became relevant in the late 19th century when two main theories arose around the evolution of mammalian molars: the tritubercular theory and the concrescence theory. The first was based on paleontological evidence and envisioned mammalian molar diversity as a series of types through which mammals progressed during evolution. The later was based on embryological evidence and saw the diversity of mammalian teeth as resulting from the ontogenetic coalescence of reptilian cone-like cusps.This paper explores the current and antecedent conceptual contributions to modern developmental evolution research on teeth in order to highlight how scientists have addressed the issue of how to explain the development and evolution of form and how these practices have changed over time.

P091 Dinusha Maheepela, University of California, Riverside
Evolutionary characterization of the *FRUITFULL* gene clade in Solanaceae
Fleshy fruits are an ecologically and economically important commodity that have evolved multiple times during the evolution of angiosperms. However, we have little knowledge on the genetic and molecular basis of fleshy fruit evolution and development. In Solanaceae (nightshades), dry fruits are plesiomorphic and fleshy fruits are derived, but the family also shows independent origins of fleshy fruit and reversion to dry. Along with the availability of multiple genome sequences and the ability

of manipulating gene function, this family thus is an ideal system for studying the evolution of fleshy fruit. FRUITFULL (FUL) genes are transcription factors that are critical for dry fruit development in Arabidopsis. Recent studies have also implicated FUL genes in fleshy tomato development. A whole genome duplication that predated the origin of Solanaceae resulted in two FUL gene clades: euFULI and euFULII. Some Solanaceae harbor two copies in each clade, suggesting further duplications. Our expression studies show the four FUL genes to be differentially expressed in dry and fleshy Solanaceae fruit, and functional data suggest a change in euFULI gene function correlated with fleshy fruit development. However the results of functional analyses, including ours, are contradictory, and euFULII remain unstudied. Our project is aimed at characterizing the evolution and function of FUL genes in Solanaceae fruit development. In addition to functional analyses, we are constructing a gene tree to pinpoint duplications and to determine if various clades are under purifying or diversifying selection.

P092 Ernesto Maldonado, National Autonomous University of Mexico (UNAM)
Evolution of Behavior in Cave Environments: Spatial Memory in *Astyanax mexicanus*
In evolution, behavior is one of the most plastic features that organisms have at hand, in order to adapt to new habitats. It is through an array of different sensory systems that they can gather information about their surroundings, colonizing even extreme environments. This is the case of the Characid fish Astyanax mexicanus with two morphotypes: Surfacefish (SF) with normal vision that inhabits rivers and blind Cavefish (CF) living in cave ponds. CF evolved in perpetual darkness and scarce food through morphological changes such as eye loss and acquisition of a greater number of larger neuromasts. It also experienced behavioral shifts as; gain of vibration attraction, and loss of traits as aggression and schooling. It is widely accepted that CF originated from SF trapped in caves. Our main question is: How Astyanax SF managed to survive in the caves? First we collected SF and CF in the wild (SEMARNAT permit 02241/13) and performed a maze experiment in complete darkness while filming with IR cameras. Spatial learning in SF has a slower learning curve than CF, however soon enough SF was completely able to form a spatial memory solving the maze in the complete absence of light.

P093 Marta Marchini, University of Calgary
The role of the growth plate in tibia length variation in mice selectively bred to increase tibia length
Mammals show a wide range of limb lengths related to their locomotion and habitat, suggesting that this trait has evolved adaptively. To understand the origins of tibia length diversity between mammals, it is essential to understand the mechanisms producing selectable variation in tibia length within a population. The main genetic and developmental processes involved in limb development are relatively well known, however, how modulation of these processes can generate bone length variation within a population is still poorly understood. To study the

genetics and development of complex skeletal traits, we have selectively bred mice for increases in tibia length (Longshanks). At generation F16, these mice show an 8% increase in tibia length versus random-bred Control mice at 14 days postnatal, and ~15% more than Control by 56 days, with differences in tibia length appearing as early as embryonic stage E14.5. The proximal tibial growth plate plays a key role in its postnatal elongation. The growth plate is a cartilage plate composed of three zones reflecting distinct stages in the chondrocyte life cycle: resting, proliferative and hypertrophic. We analyzed the proximal tibial growth plate of 14-day old Longshanks and Control mice using histomorphometry and cell proliferation assays. Preliminary data shows that in Longshanks, the growth plate is ~20% thicker than control. The hypertrophic zone is larger, and has more cells in Longshanks, which suggests that rate of transition from proliferating to hypertrophic chondrocyte plays an important role in producing tibia length variation.

P094 Jeffrey Marcus, University of Manitoba

Report of an additional A-P developmental compartment boundary and organizer in the far posterior of butterfly and Drosophila wings

We evaluated the organizational effects of compartment boundaries in butterflies and fruit flies. First, Independent Contrast Analysis (ICA) analysis was applied to eyespot patterns in Vanessa butterflies (Lepidoptera: Nymphalidae). ICA of eyespot color elements revealed significant positive correlations between eyespots 2 and 5 and between eyespots 3 and 4 on all wing surfaces. Similar patterns of correlation between these eyespots are known from some wing surfaces in both Junonia and Bicyclus butterflies, suggesting that this nested pattern of symmetry on either side of vein M3 may occur across the family Nymphalidae. This line of symmetry is distinct from and posterior to the compartment boundary and wing organizer system defined by Engrailed expression and dpp signaling (between veins M1 and M2). Surveying the wing cell color patterns across all families of butterflies reveals a similar nested set of A-P color pattern symmetry in this region of the butterfly wing. Evaluation of spontaneous Lepidopteran mitotic clones reveals a peak abundance of clonal boundaries between wing cells 2 and 3, which is consistent to the presence of a compartment boundary in this vicinity. Finally, in FLP/FRT wing clones produced in Drosophila, there is a clonal boundary posterior to the L5 wing vein that is homologous to the M3 vein dividing wing cells 3 and 4 in butterflies. Collectively, these findings suggest the existence of both a previously undetected additional compartment boundary and a new A-P wing pattern organizer near vein M3 that is responsible for patterning the posterior portion of holometabolous insect wings.

P095 Vladimir Mashanov, University of Puerto Rico

Glial cell behaviour in echinoderm neural regeneration

Echinoderms quickly regenerate various parts of their body, including the central nervous system (CNS). The phylum Echinodermata together with hemichordates constitute an outgroup to chordates. This phylogenetic position makes them equally suitable as subjects of

fundamental research addressing the evolution of neural regeneration in deuterostomes and as a source of insights into how CNS regeneration can be improved in mammals. The echinoderm CNS is composed of a neuroepithelium, whose supporting framework is formed by radial glial cells strongly resembling radial glia of chordates in their morphological and immunocytochemical characteristics. The post-injury response in the echinoderm CNS involves extensive dedifferentiation of the radial glia. Dedifferentiated glial cells become highly proliferative and form a tubular outgrowth across the wound gap, which later becomes repopulated with neurons. The reaction of echinoderm glia to injury thus sharply contrasts with formation of the inhibitory glial scar in the lesioned mammalian CNS. Our transcriptomic analysis identified Myc as the only over-expressed pluripotency factor in the injured echinoderm CNS. In situ hybridization showed expression of this gene in the radial glia, and RNAi-mediated knockdown demonstrated that Myc was required for proper dedifferentation of the glia and for triggering programmed cell death in response to injury. So far, the progress in understanding molecular mechanisms driving echinoderm regeneration has been hampered by the lack of functional genomic tools. Our study is the first implementation of RNAi methodology in adult echinoderms, and Myc is thus the first transcription factor, whose role in echinoderm regeneration was experimentally established.

P096 Svetlana Maslakova, University of Oregon

Origin of a novel larval body plan in ribbon worms (Nemertea, Spiralia)

Nemerteans are a phylum of marine worms, and members of the Spiralia — the most diverse clade of bilaterian animals, but the least studied in terms of developmental mechanisms. Among bilaterians, nemerteans are especially suitable for studies of body plan evolution because a novel larval body plan (the pilidium), evolved within the phylum. The pilidium is a long-lived planktonic larva with unique morphology and development, and catastrophic metamorphosis. The juvenile nemertean develops inside the pilidium from a set of invaginated rudiments, called imaginal discs, and, once complete, erupts from and devours the larva. While many marine invertebrates develop indirectly via a larval form distinct from the adult, in most such cases (e.g. echinoderms) indirect development is considered ancestral to the phylum. The nemertean pilidium, on the other hand, evolved in a single clade, the Pilidiophora, from a more direct development found in other nemertean taxa. This allows comparisons between maximally-indirect development (via pilidium) and direct development within a single phylum which, we hope, will help us understand how a novel larval body plan arose. Here I will share our insights into the developmental and evolutionary origin of the pilidium larva, gained from studies of larval morphology, cell lineage, cell proliferation, and gene expression in nemertean development. In particular, I will focus on how pilidiophorans modified conservative spiralian developmental program to build the pilidial larval body as well as the juvenile inside.

P097 John Masly, University of Oklahoma

The evolution of novel sex-specific traits: Dissecting the genetics and development of genital morphological variation in Drosophila

Understanding the evolutionary forces that drive morphological differences among species and how these forces shape molecular variation that directs developmental differences are major goals of evolutionary developmental biology. External genital structures display one of the more striking morphological differences observed among sister species, and this variation in morphology is thought to be shaped primarily by sexual selection. However, little is known about how sexual selection shapes variation at the molecular and developmental levels to give rise to such widespread, rapid morphological evolution. We genetically modified the size and shape of two newly evolved male secondary genital structures in Drosophila, the posterior lobes of the genital arch, both within and between species to identify the selective forces responsible for affecting variation in genital morphology. We found that the posterior lobes are necessary for copulation and that they also appear to be targets of multiple post-copulatory sexual selection processes that shape quantitative variation in morphology. Using a collection of interspecific genetic introgression lines, we mapped several regions of the genome that specify species-specific morphologies and have identified a novel gene that specifies variation in posterior lobe size. Interestingly, this gene is not a major transcriptional regulator, but instead appears to be a signaling peptide whose expression level specifies size variation. We are currently combining functional genetic tests with newly developed live-cell imaging approaches to characterize how variation at this locus— and others within the posterior lobe gene regulatory network— directs developmental differences between species.

P098 Yuji Matsuoka, The University of Tokushima

Functional analysis of a Hox gene, abdominal-A, using CRISPR/Cas9 system in the cricket *Gryllus bimaculatus*

An orthopteran insect, the cricket Gryllus bimuculatus, is a model system for studying genetic mechanisms of embryogenesis and regeneration. Recently, we have established techniques of gene knock-out via genome editing using ZFNs, TALENs, and CRISPR/Cas9 system. However, gene knock-in has not yet been achieved in this species. We tried targeted gene knock-in in the cricket via homology-independent DNA repair using the CRISPR/Cas9 system. We constructed donor vectors containing an eGFP expression cassette. We chose a Hox gene, abdominal-A (abd-A), as a target. In injected embryos (G0), mosaic eGFP expression was observed in the abdominal region (33%). In addition, embryos showed two different types of phenotypes. One is the formation of appendage-like structures in the ventral side of abdomen (10% of injected embryos), which is similar to the abd-A phenotype in the short germ insect Tribolium. The other is loss of abdominal segments (9%), which has not been reported so far. At least 14% of the fertile G0 adults produced G1 embryos with eGFP expression. These results indicate that we have succeeded in targeted gene knock-in in the cricket. Our method makes it easy to isolate

homozygous or heterozygous mutants without PCR-based genotyping. By crossing the eGFP positive G1 crickets, we should be able to obtain homozygous mutants. We will show further details of the progress in our functional analysis.

P099 David Matus, Stony Brook University
Cell division and cell cycle exit: An "elegant" mechanism regulates nematode uterine-vulval morphogenesis
Comparative studies of the nematode vulva have long been used as a model for understanding the evolution of cell fate specification, induction and cryptic variation. We are using this system to understand the evolution of morphogenesis. We have examined the process of uterine-vulval attachment during development across 21 species of rhabditid nematodes. We find that a non-dividing vulval cell always stabilizes the basement membrane gap that forms between these organs. Through a combination of cell cycle manipulation and live cell imaging in Caenorhabditis elegans we show that vulval cell division facilitates expansion of this breach by promoting basement membrane movement. This is balanced by targeted cell-cycle arrest, which halts basement membrane movement precisely limiting the size of the gap opening. This gap expansion is directed by an enrichment of the basement membrane component laminin, which accumulates at the gap edge and promotes increased integrin levels in arrested vulval cells. Taken together, these studies reveal a novel mechanism, conserved across several hundred million years of evolution, which has been used to regulate the size of a basement membrane gap, controlling the exchange of cells between tissues.

P100 Christine Mayer, University of Oslo
Modeling Genotype-Phenotype Maps by combining a multilinear model with Boolean Networks
Evolvability and robustness are crucial concepts to understand evolutionary processes. The relationship between them helps us to understand evolutionary change and the origination of evolutionary innovations. The genotype-phenotype map is a useful concept to study the relationship between evolvability and robustness, but how these are connected is a still unanswered question subject to debate. By using a model based on a multilinear framework combined with the idea of Boolean networks, we simulate different genotype-phenotype maps using logical Boolean operations. We demonstrate that the relationship between evolvability and robustness is dependent on the definition of the underlying genotype-phenotype map. The correlation between evolvability and robustness can be positive as well as negative, depending on the complexity of the underlying genotype-phenotype map based on logical Boolean operations.

P101 Anyi Mazo-Vargas, Cornell University
The Developmental Basis of Wing Color Pattern in Monarch Butterflies
Animal color patterns are a conspicuous example of morphological variation and adaptation. Butterflies in particular display striking wing

patterns that are vital for ecological and behavioral functions such as mate choice, camouflage, mimicry, and warning signaling. Monarch butterflies (*Danaus plexippus*) in particular are perhaps the most famous textbook example of warning coloration (aposematism), which is manifested in the form of spectacular orange and black wing patterns. Little is known, however, regarding the developmental genetics of color patterning in *D. plexippus*. Here, we report expression patterns for two critical developmental patterning genes, *optix* and *WntA*, both of which have been associated with red and black wing pattern evolution in *Heliconius* butterflies. We used *in situ* hybridization, immunohistochemistry, pharmacological assays, and functional gene knockout (CRISPR) to test genotype – phenotype associations. Our results indicate these genes are pivotal in the development of the striking wing color patterns observed in *D. plexippus*. Overall, this study serves both as an important starting point to understand the genetic basis of wing color pattern development in the Monarch butterfly, and also as a case study for understanding the mechanism by which a toolset of genes is recruited in the development of warning coloration in butterflies.

P102 Meghan McKeown, University of Vermont

Evolution of Vernalization Responsiveness in the Temperate Grass Subfamily Pooideae

The ability of plants to match their reproductive output with favorable environmental conditions has major consequences both for lifetime fitness and geographic patterns of diversity. In temperate ecosystems, some plant species have evolved the ability to use winter chilling (vernalization) as a cue to ready them for spring flowering, a trait known as vernalization responsiveness. However, it is unknown how important the evolution of vernalization responsiveness has been for the colonization and subsequent diversification of taxa within the northern and southern temperate zones. Grasses of subfamily Pooideae, including several important crops such as wheat, barley, and oats, predominate in the northern temperate zone, and it is hypothesized that their radiation was facilitated by the early evolution of vernalization responsiveness. Predictions of this single origin hypothesis are that a response to vernalization is widespread within the subfamily, and that the genetic basis of this trait is conserved. To test these predictions, we compared flowering time and expression of wheat vernalization gene (VRN1 and VRN3) orthologs in phylogenetically representative pooids under chilling and control conditions. Our data demonstrate that vernalization responsive pooids are widespread, and at least part of the vernalization gene network is conserved, throughout the subfamily. These results thus support the idea that the evolution of vernalization responsiveness was important for the initial transition of pooids out of the tropics and into the temperate zone.

P103 Shaadi Mehr, State University of New York & American Museum of Natural History

New Family of Fluorescent Proteins in Vertebrates: Adaptive Evolution of Fatty Acid Binding Proteins That Produce Bright Fluorescence in the Marine Environment

We report the identification of a family of bilirubin-inducible fluorescent proteins (FPs) from marine eels and demonstrate a key region of the sequence that serves as an evolutionary switch from non-fluorescent to fluorescent fatty acid-binding Proteins (FABPs). Transcriptomes of two brightly fluorescent chlopsid (Kaupichthys) eels were sequenced and assembled, and two new FPs were identified, cloned and characterized (ChlopsidFP-I and II). Phylogenetic analysis of 176 FABPs, spanning 16 vertebrate classes, shows that the fluorescent FPs diverged as a new protein family and are the sister group to brain FABPs. Our results indicate that the evolution of this family involved at least three gene duplication events. Fluorescent FABPs possess a unique, conserved tripeptide Gly-Pro-Pro sequence motif that has undergone intense positive evolutionary selection. This sequence appears essential for fluorescent induction by bilirubin. The emergence of this insertion in FABPs is restricted exclusively to fluorescent eels and is not present in any other FABP from terrestrial or marine organisms. This work supports the hypothesis that the unique spectral characteristics of the ocean are driving the evolution of FP families from those that are non-fluorescent to those that emit visible fluorescence when excited by blue light. ChlopsidFPs exhibit a blue-shifted fluorescence emission spectra compared to UnaG, a fluorescent FABP from the eel Anguilla japonica, suggesting the potential to modulate the fluorescence via mutations of the amino acid sequence. The discovery of this new class of FPs with diverse properties provides new templates for the development of protein- based fluorescent tools.

P104 Paul Minor, Hopkins Marine Station of Stanford University

Evolution of Retinoid Signaling in Deuterostomes: Insights from the Hemichordate *Saccoglossus kowalevskii*

Retinoic acid is a powerful morphogen with many important functions in chordate development. It plays critical roles in anteroposterior patterning and organogenesis as well as modulating cell survival, proliferation, and differentiation. There has been extensive research on the roles of retinoid signaling in vertebrate development as well as basic comparative developmental studies in invertebrate chordates. Recent work shows the presence of some pathway components outside of deuterostomes; however, the evolution of this signaling pathway in metazoan body plan development remains unclear due to the lack of functional data outside of chordates, specifically within echinoderms and hemichordates. We have identified all core components of the retinoic acid signaling pathway in the hemichordate *Saccoglossus kowalevskii* and described the expression pattern of each gene using *in situ* hybridization. The presence of both all-trans and 13-cis retinoic acid in the embryo and adult has been confirmed through mass spectrometry. We have tested the extent of pathway autoregulation, a hallmark of retinoic acid signaling in chordates, and function through exogenous

treatment of all-trans retinoic acid and siRNA knockdown of pathway components coupled with qPCR and *in situ* hybridization analysis. Finally, to better understand how this pathway behaves on the cellular level we have utilized *in vitro* protein biochemistry to test homo- and hetero-oligomerization capabilities of the nuclear hormone receptors, RAR and RXR, as well as the ligand binding capacities of each receptor. Our results demonstrate a role for retinoic acid signaling in *S. kowalevskii* and provide insight into the evolution of this pathway in deuterostomes.

P105 Tetsuto Miyashita, University of Alberta

Fishing for jaws in early vertebrate evolution: A new hypothesis of mandibular confinement

The evolutionary origin of the vertebrate jaw persists as a deeply puzzling mystery. More than 99% of living vertebrates have jaws, but the evolutionary sequence that ultimately gave rise to this highly successful innovation remains controversial. A synthesis of recent fossil and embryological findings offers a novel solution to this enduring puzzle. I propose that the jaw evolved via spatial confinement of the mandibular arch (the most anterior pharyngeal arch within which the jaw arose). Fossil and anatomical evidence reveals: (a) the mandibular region was initially extensive and distinct among the pharyngeal arches; and (b) with spatial confinement, the mandibular arch acquired a common pharyngeal pattern only at the origin of the jaw. The confinement occurred via a shift of a domain boundary that restricted the space the mesenchymal cells of the mandibular arch could occupy. As the surrounding domains replaced mandibular structures at the periphery, this shift allowed neural crest cells and mesodermal mesenchyme of the mandibular arch to acquire patterning programs that operate in the more posterior arches. The mesenchymal population within the mandibular arch was therefore no longer required to differentiate into specialized feeding and ventilation structures, and was remodelled into a jaw. Fossil evidence documents a general trend of such mandibular confinement from jawless to jawed taxa. Embryological evidence corroborates that the mandibular arch must be spatially confined for a jaw to develop. This new interpretation suggests neural crest as a key facilitator in correlating elements of the classically recognized vertebrate head 'segmentation'.

P106 Patricia Moore, University of Georgia

Conservation of pleiotropic effects of Broad Complex in an evolutionary trade-off in the burying beetle *Nicrophorus vespilloides*

Resorption of oocytes and reallocation of resources towards survival provides the opportunity to adaptively respond to a variable environmental. While oosorption has been studied in an ecological context, little is known about the molecular mechanisms underlying the decision to resorb oocytes. In Drosophila melanogaster the genetic switch point between oocyte death and development under varied food environments relies on differential expression of Broad Complex (BRC) isoforms. BRC has a conserved role in larval development but is the

role of BRC in mediating reproductive plasticity also conserved? We predicted that conservation in development would lead to conservation in ancillary roles as well. To test this, we investigated how BRC is involved in resorption in the burying beetle, Nicrophorus vespilloides, by examining the molecular response to removing the resource required for reproduction from females. This work represents an example of how developmental mechanisms can inform our understanding of evolutionary trade-offs.

P107 Thomas Morgan, University of California, Berkeley

How Plasticity can Re-direct Evolution

In the 1896 paper "A New Factor in Evolution", James Baldwin proposed "organic selection" as a mechanism by which development could influence evolution. Now commonly referred to as The Baldwin Effect, it is generally understood to describe the genetic accommodation of plastic traits via selection. However, when initially proposed, it also included the idea that plasticity, by shaping phenotypic variation, could also determine the course of evolution. This latter notion remains controversial, conflicting with the Modern Synthesis view of selection as the sole guiding force in evolution and that evolution can be understood independently of development. Here we present a series of evolutionary simulations that support both functions of organic selection. First we explore genetic accommodation and show that in a stable environment selection will preferentially accommodate traits that are difficult to acquire, have greater fitness benefits, and affect the acquisition of other traits. Secondly we show that the course of evolution can be changed by biases in plasticity without the need for the inheritance of acquired characters. Such plasticity can reverse the direction of selection and causes the genetic accommodation of phenotypes favored by plasticity over those that would be favored by selection in the absence of plasticity. We use these findings to offer a novel explanation for why complex cognition is unusual and discuss candidate cases of organic selection. We conclude that organic selection is likely to be a widespread phenomenon and that the inclusion of developmental plasticity in an evolutionary framework would be of benefit.

P108 Yuuta Moriyama, Institute of Molecular and Cellular Biosciences

Neofunctionalization of elastin demarcates teleost heart by regulating cell fate through mechanotransduction

The evolution of novelty is a key process in the diversification of life, yet investigation about genetic changes providing "novelty" remains one of the greatest challenges in the field of biology. Teleost have evolutionary novelty "Bulbus arteriosus (BA)" which is a specialized organ seen in the outflow tract (OFT) of the heart. BA have the important role of acting as a "windkessel" organ, absorbing the energy of the bolus of blood ejected from the ventricle by elastic expansion and recoil, and smoothing the pressure wave down to the arterial tree. The BA is a unique organ that is composed of smooth muscle while the OFTs in other vertebrates including non-teleost fish are composed of myocardium. Despite its importance, the mechanism underlying development and evolution of BA formation is largely unknown. Here we

show that teleost-specific extracellular matrix (ECM) gene, elastinb (elnb), was generated by teleost-specific 3 rounds whole-genome duplication (3R WGD) and subsequent functional divergence occurred on elnb and elna (neofunctionalization) contributes to acquisition of BA by regulating cell fate determination of cardiac precursor cells into smooth muscle. Taken together, our study clearly illustrates that WGD and subsequent neofunctionalization contributes generation of phenotypic evolutionary novelty under natural selection and 3R WGD demarcates infraclass Teleostei. Furthermore, our findings uncover a novel mechanism of cell fate determination of cardiac precursor cells by ECM through mechanotransduction.

P109 Jacqueline Moustakas-Verho**,** Institute of Biotechnology, University of Helsinki

Predicting the role of mechanical forces in tooth morphogenesis

The mammalian dentition is a model system in which to study evolutionary and developmental mechanisms of pattern formation. Teeth develop through an epithelial-mesenchymal interaction between oral ectoderm and neural crest-derived mesenchyme. Tooth shape is controlled by embryonic signaling centers, the enamel knots, via differential growth and folding of the epithelium. Enamel knots form at the places of future cusps, the prominences that are the basic units of the tooth shape. A computational model of development that combines parameters of genetic and cellular interactions predicts a mechanical role for the mesenchyme in the generation of cusps in mammalian molar teeth. Using computed tomography, we measured the density of mesenchyme as a proxy for mechanical force by the mesenchyme on the dental epithelium. Three-dimensional reconstructions of the embryonic teeth show differences in mesenchymal density that predict epithelial morphogenesis and the formation of tooth cusps. We then tested these density differences in dental mesenchyme using confocal microscopy and physical models.

P110 Keshava Mysore, Indiana University School of Medicine-South Bend

Comparative Analyses of Olfactory Development in Dipteran Insects

Hematophagous mosquitoes, which vector many tropical diseases, are well adapted dipteran insects that diverged ~250 million years ago from *Drosophila melanogaster,* a well-characterized genetic model organism. While the olfactory system of *D. melanogaster* is now one of the most thoroughly studied sensory systems, much less is known about organization and development of the mosquito olfactory system, which plays a prominent role in host-seeking behavior and subsequent pathogen transmission. Mosquito host-seeking behavior is based on chemosensation involving the peripheral olfactory and gustatory sense organs, as well as the central olfactory and gustatory circuitry of the brain. To further understand the cellular processes involved in development and organization of the mosquito olfactory system, we systematically studied and compared post-embryonic olfactory system development in *Aedes aegypti* (dengue and yellow fever vector) and *Anopheles gambiae* (principal African malaria vector) and compared it

to that of *D. melanogaster.* These studies were facilitated by *Drosophila* cross-reacting antibodies that label epitopes in the developing *A. aegypti* and *A. gambiae* olfactory systems. Our investigation revealed both prominent differences and remarkable similarities in the organization of mosquito post-embryonic olfactory systems with respect to *D. melanogaster*. Understanding the developmental origins of mosquito olfactory systems will facilitate ongoing functional genetic studies and further our understanding how responses to various chemicals have evolved during development of different insect species.

P111 Tetsuya Nakamura, University of Chicago

The Mechanisms Controlling Ancient Fin Development

Classical comparative studies indicate that the loss of the anterior portion of the fin is one of the most remarkable transitions from fish fins to tetrapod limbs. The paired fins of primitive fish have three basal endoskeletal structures, the pro-, meso- and metapterygium. However, in crown Sarcopterygians the anterior two structures, the pro- and mesopterygium, disappeared, resulting in the establishment of metapterygial axis and acquisition of superior maneuverability. Whereas the toolkit genes of tetrapod limb development have been well characterized in mammals, developmental and evolutionary mechanisms controlling appendage width and fin configuration in ancestral fish have been unexplored. Here we report on the ancient developmental mechanisms of paired fins in skate (Leucoraja erinacea; Chondorichthyes), which has three basal bones and is regarded as an basal fin state.

P112 Nagayasu Nakanishi, University of Queensland

An ancient role for Notch in the evolution of cellular transformation in animals

In animals, the ability of differentiated cells to change their cellular states is critical in development, regeneration and oncogenesis, but the origin and evolution of cell transformation mechanisms are enigmatic. Notch signaling regulates diverse cellular transformations across eumetazoans (vertebrates, sea anemones and allies), indicative of an early evolutionary origin. Here we show in the demosponge Amphimedon queenslandica that Notch activity is required for the transformation of two epithelial cell types—larval sensory cells at metamorphosis and juvenile choanocytes during growth—into a common mesenchymal stem cell. Interestingly, we find a novel, ecologically regulated mode of Notch activation in the larval sensory cells, whereby Ca2+-mediated metamorphic signal transduction controls actin-driven cell shape changes necessary to activate Notch receptors. These results support an ancient role for Notch in conversion of cellular states, predating the origins of sponges and eumetazoans, and suggest that cellular transformation can evolve by deploying distinct Notch activation mechanisms.

P113 Scott Nichols, University of Denver

Cellular innovations for adhesion in the animal stem lineage

The body plan of sponges (phylum Porifera) is an outlier among modern animals and is thought to have special evolutionary significance. Sponges lack muscles, nerves and a gut. Instead they are composed of few cell types and simple tissues that function to pump water through an internal canal network where bacterial prey are filtered by a specialized tissue called the choanoderm. Sponges represent an early evolutionary branch of animals and there is striking similarity between cells of the sponge choanoderm and choanoflagelaltes, the unicellular relatives of animals. Our research examines the evolutionary significance of the sponge body plan by focusing on the cell and developmental biology of the choanoderm. Our initial strategy has been to examine the function of sponge homologs of the classical cadherin/catenin complex, which is the primary adhesion mechanisms operating in bilaterian tissues. Preliminary data suggest significant structural differences between sponge tissues and bilaterian epithelia. We complement this candidate gene approach with a comprehensive analysis of gene expression in the sponge choanoderm. Collectively, these data contribute to a new understanding of how and when the CCC evolved, and indicate a number of exciting new candidates for understanding how the unicellular ancestors of animals were modified during the transition to multicellularity.

P114 Mark Nielsen, University of Dayton

Tubulin Evolution in Holometabolus Insects

Tubulins are a family of eukaryotic structural genes that form microtubules, fundamental components of the cytoskeleton that mediate cell division, shape, motility, and intracellular trafficking. Previous in vivo studies in Drosophila find a stringent relationship between tubulin structure and function. This raises an important question, How do tubulins evolve while maintaining their function? To answer, we use molecular evolutionary analyses to characterize the evolution of insect tubulins. Sixty-six alpha tubulins and eighty-six beta tubulin gene copies were retrieved and subjected to molecular evolutionary analyses. Four ancient clades of alpha and beta tubulins are found in insects, a major isoform clade (alpha 1, beta 1) and three minor, tissue-specific clades (alpha 2-4, beta 2-4) generated through gene duplication events on major beta and alpha tubulin ancestors. Strong purifying selection acts on all tubulins, and show little sequence variation except in the last 15 carboxy terminus tail (CTT) residues. CTT residues overwhelming comprise the co-evolving residues between Drosophila alpha 2 and beta 3 tubulin proteins. Compensatory evolution is found in Drosophila beta 2 tubulin cis-regulation, and reveals selective pressures acting to maintain testis expression without the use of previously identified cis-regulatory elements. These results show that tubulins evolve through gene duplication, followed by subfunctionalization of expression domain, and/or divergence of coding sequence CTT residues, resulting in the tissue-specific minor insect isoforms, including the highly diverse α3, α4, and β2 reproductive isoforms, illustrating that even a highly conserved protein family can participate in the adaptive process.

P115 Kara Nordin, Northwestern University

Shared regulatory programs suggest retention of blastula-stage potential in neural crest cells

Neural crest cells, unique to vertebrates, arise in the ectoderm but can generate cell types typically categorized as mesodermal. This broad developmental potential persists past the time when most ectoderm-derived cells become lineage restricted. The ability of neural crest to contribute mesodermal derivatives to the bauplan has raised questions about how this apparent gain in potential is achieved. Here we describe shared molecular underpinnings of potency in neural crest and blastula cells. We show that key neural crest regulatory factors are expressed in blastula animal pole cells and are essential for the developmental potency of these cells, and that establishing a neural crest state retains the ability of ectoderm derived cells to contribute to all three germ layers past when they should have become lineage restricted. We suggest that neural crest cells may have evolved as a consequence of a subset of blastula cells retaining activity of the regulatory network underlying pluripotency.

P116 Rodrigo Nunes da Fonseca, Universidade Federal do Rio de Janeiro

The pioneer transcription factor zelda is required for growth zone patterning and metamorphosis in the beetle *Tribolium castaneum*

Gene regulatory networks (GRN) result from the evolution of transcription factors and the cis-regulatory modules (CRMs) they bind to. The zinc-finger transcription factor zelda (zld) is essential for maternal zygotic transition (MZT) in Drosophila melanogaster, when it directly binds over thousand CRMs and regulates chromatin accessibility. Drosophila displays a long germ type of embryonic development, where all segments are simultaneously generated along the whole egg length. It remains unclear if zld is also involved in MZT of basal groups (e.g. short-germ insects) or in other biological processes. Here, we provide the first biochemical, morphological and computational analysis of zld in the short-germ beetle Tribolium castaneum (Tc). Computational analyses identified segmentation, metamorphosis and wing formation as putative new biological roles of zld in Tribolium. mRNA expression and knockdown during several developmental stages (embryo, larvae, pupae and adult) confirmed that Tc-zld is essential not only for MZT, but also for the aforementioned processes in short-germ insects. These results imply that the pioneer transcription factor zld is not only involved in MZT but also in other key developmental processes. We discuss the implications of these findings for the evolution of gene regulatory networks in arthropods.

P117 Pinar Onal, New York University

Step-by-Step Evolution of Bcd's Anterior Patterning Functions

How new protein functions evolve is a fundamental question in biology. The bicoid (bcd) gene emerged from a gene duplication, which also gave rise to the paralogous gene, zerknullt (zen). Bcd evolved rapidly and diverged extensively from Zen in sequence and function. It is transformed into a key regulator of anterior patterning in Drosophila, due

most probably to newly gained features, such as anterior localization, a shift in DNA-binding specificity, and the acquisition of the ability to bind to RNA. The Bcd Homeodomain (HD) is necessary for Bcd's patterning functions via regulating transcription and translation of its target genes. Moreover, it can confer most of Bcd's target regulation activities when inserted into the Zen protein. Preliminary data also suggest that single amino acid changes in the HD can have dramatic consequences. In order to understand how Bcd HD gained new and critical functions, we mimic predicted evolutionary steps starting from an ancestral Zen-Bcd (Zba) HD, which is inferred using a maximum likelihood-based ancestral protein reconstruction method. In the immediate future, we will investigate how evolutionary innovations conferred on Bcd the ability to bind DNA, RNA, and to pattern Drosophila embryo.

P118 Duygu Ozpolat, CNRS

Developing live-imaging tools for investigating cell division and cell cycle patterns in the *Platynereis dumerilii*

Platynereis is a marine annelid (segmented worm) which develops from an embryo into a 3-segmented larva, and then into a juvenile with many segments before reaching sexual maturity. During its various developmental stages, the worm forms and then continuously adds new segments (or regenerates them upon injury) from its posterior end. A stem cell population called teloblasts is involved in producing tissues of each newly formed segment, but the division and cycling patterns of this cell population is still largely unknown. Currently, most of our knowledge about developmental cell division and cell cycle dynamics comes from methods that provide only static images (snapshots) from fixed samples like immunohistochemistry for cell division markers or thymidine analog labelling techniques (such as BrdU, and EdU). Moreover, the necessity of sample fixation imposes significant limitations to observing cell cycle dynamics. Here we present our ongoing work to visualize cell division and cell cycle patterns in Platynereis, in real-time, using in-vitro transcribed mRNA injections of fluorescently-labeled histone and membrane constructs, and transposon-mediated transgenesis techniques for a cell cycle reporter (the fluorescent ubiquitination-based cell cycle indicator: FUCCI). We expect that these techniques will enable us to address questions such as whether cycling of teloblasts is linked to segment formation, and whether they divide synchronously or random. These findings will also help clarify the role of cell cycle machinery in establishment and/or maintenance of the stem cells in development.

P119 Nesibe Ozsu, National University of Singapore

The origin of the eyespot gene network in *Bicyclus anynana* butterflies

The molecular basis for the origin of novel traits is largely unknown. We propose that this type of exploration can begin with the identification of the trait's underlying gene regulatory network, Butterfly eyespots are complex novel traits that originated once within the family Nymphalidae. Although several genes have been discovered associated with eyespot development, the identity of the novel gene network remains

unidentified. Here, Next-Gen sequencing and transciptome analyses were used to identify a large set of genes associated with eyespot development at 3hrs after pupation, during the early signaling stages that lead to the differentiation of the color rings. The total set of genes involved in eyespot development were identified using comparative transcriptomics of homologous micro-dissected tissue of wings that either develop or don't develop eyespots. We identified 277 genes that were significantly up-regulated and 318 genes that were significantly down-regulated in wing tissue that develops eyespots. Several of the differentially expressed genes are currently being confirmed for eyespot-specific expression using in situ hybridizations with labeled probes.

P120 Nipam Patel, University of California, Berkeley
Compartment Boundaries in Lepidopteran Wings
The existence of an anterior-posterior compartment boundary has been well documented in the model insect, Drosophila melanogaster, and the compartment boundary in Drosophila is thought to play an important role in maintaining the Decapentapalegic signaling center. By analyzing the patterns of approximately three thousand gynandromorph Lepidoptera, we present evidence that the wings of butterflies and moths have three compartments, with the middle compartment located between the M1/M2 veins. One butterfly compartment is equivalent to the P compartment of Drosophila, but it is the A compartment of Drosophila that is subdivided into two compartments (A1 and A2) in Lepidoptera. We also show that this three compartment subdivision of the wing, or at least a remnant of this subdivision, is actully present in Drosophila, and can be visualized through the generation of clones during early Drosophila embryogenesis. We conclude that the subdivision of the insect embryo segment into three compartments leads to the subdivision of the wing, and other appendages, into three compartments.

P121 Michael Perry, New York University
Butterfly color vision: Stochastic patterning mechanisms and increased sensory receptor diversity
Butterflies use color vision extensively to navigate the natural world. Their retinas are more complex than those found in Drosophila, where development and patterning has been heavily studied. Instead of the eight photoreceptors found in flies, butterflies have an additional ninth photoreceptor per ommatidium ("unit eye"). They also have three main types of ommatidia instead of the two distributed stochastically in the fly retina. We set out to determine how butterflies generate increased sensory receptor diversity to provide improved color vision, and how much of the retinal patterning network from Drosophila they reuse. We show that the regulatory network that defines photoreceptor subtypes in Drosophila is redeployed in butterflies (Papilio xuthus and Vanessa cardui) to generate additional subtypes. The R7 photoreceptor marker Prospero is expressed in two rather than one photoreceptors per ommatidium. In Drosophila, a stochastic decision to express the transcription factor Spineless in R7 determines which of two subtypes of

ommatidia is specified. CRISPR knock-out of Spineless in butterflies shows that Spineless also controls stochastic choice in each of the two R7s, suggesting a deep evolutionary conservation of stochastic patterning mechanisms. Having two stochastically distributed types of R7s allows for specification of three ommatidial types instead of two, which in turn allowed for the evolution and deployment of additional opsins, tetrachromacy, and improved color vision. These efforts provide evidence that our extensive knowledge of patterning in the Drosophila visual system applies to other groups, and that adaptation for specific visual requirements can occur through modification of this network.

P122 Daniel Pers, University of Illinois at Chicago

A comprehensive characterization of a non-*Drosophila* dorsoventral patterning GRN provides new insights into regulatory network evolution

Gene regulatory networks are vital for developmental processes such as patterning and morphogenesis. Modifications to theses networks allow for the emergence of novel developmental outputs, thus exploring how GRNs vary across phylogenies can provide insight on the evolution of development. The dorsoventral GRN of the *Drosophila melanogaster* embryo is one of the most well characterized examples of these networks; however, it is highly derived and not representative of most insects. The wasp, *Nasonia vitripennis*, undergoes a similar mode of embryogenesis, and just prior to gastrulation has a nearly identical spatial expression of tissue specific marker genes, but establishment of these patterns is quite divergent. Whereas *Drosophila* relies solely on Toll signaling, *Nasonia* exhibits a reduced role of Toll, instead utilizing BMP as its global patterning signal. These observations, in conjunction with the availability of genetic/molecular tools establish *Nasonia* as a prime candidate for comparative GRN analysis. Candidate genes with differential expression along the dorsoventral axis were uncovered utilizing RNAi-RNAseq. *In situ* hybridization confirmed nearly 100 genes have significant differential expression patterns along the DV axis and allowed comparisons to know patterns of *Drosophila* orthologs. Using this approach, we uncovered a set of genes with conserved dorsoventral patterning roles between the two species and a set specific to each species. Within these a number of interesting subsets emerged: *Drosophila* patterning genes with reassigned tissue specific expression in *Nasonia*, novel *Nasonia* dorsoventral genes lacking *Drosophila* orthologs, and dorsoventral genes that appear to have arisen from a series of bacterial/viral lateral gene transfers.

P123 Timothy Peterson, University of Vienna

Novelty origination through threshold events: Examining fish pharyngeal jaws using finite element analysis

Qualitatively discontinuous evolutionary changes, such as the introduction of novel traits into a bodyplan, can originate as threshold events from quantitative changes within a developmental system. These arise most commonly from alterations of the genome and are expressed in modifications of processes such as cell signaling, reaction-diffusion patterning, or biomechanics. This signifies that phenotypic novelties are

the result of interactions and rules present in development and are not random events despite their discontinuous origin. Therefore, novel traits illustrate how development has generative potential and how EvoDevo has explanatory force. Quantitative, measurable events lead to qualitative and discontinuous origins. We demonstrate a new method for how to proceed by tracking the biomechanics of the pharyngeal jaw apparatus during the ontogeny of cichlid fishes using finite element models created from microCT images of radiopaque stained specimens. Our results show that a simple change in tissue configuration between the epibranchials 4 and the pharyngobranchials yields large differences in compressive force between the upper pharyngeal jaws and the neurocranium, which is responsible for eliciting novel cartilage formation.

P124 John Postlethwait, University of Oregon

IcemiR: The evolution of microRNA control in Antarctic fish

microRNAs (miRNAs) are key post-transcriptional regulators of gene expression that modulate development and physiology in temperate animals. miRNAs depend on temperature-senstive RNA:RNA interactions but have yet not been studied in Antarctic animals, including notothenioid fish, which dominate the Southern Ocean. We compared miRNA regulation in Antarctic vs. temperate fish to learn the roles of miRNA regulation in adaptation to constant cold; and in bottom-dwelling red-blooded nototheniods vs. high buoyancy white-blooded icefish to understand miRNA regulation after loss of hemoglobin genes and red blood cells, enlarged heart and vasculature, and increased buoyancy, which arose by decreased bone mineralization and increased lipid deposition. Results revealed evolution of miRNA gene sequences, expression patterns, and genomic content in Antarctic fish related to their evolved traits. Evolution of these developmental regulators was likely highly integrated with, and perhaps necessary for, survival as the Southern Ocean cooled to its current frigid state.

P125 Kara Powder, University of Massachusetts, Amherst

Constraint and diversification of developmental trajectories in cichlid facial morphologies

Development has a dual role in morphological evolution. It can generate phenotypic variation on which natural selection can act, but also constrain the spectrum of phenotypes that can be produced. East African cichlids exhibit an extensive adaptive radiation, and pivotal to this are species-specific craniofacial structures that allow ecological specialization. To understand the ontogenetic origins of this morphological variation, we examined the developmental trajectories among six species of Lake Malawi cichlids (n500 individuals) that span a major ecomorphological axis. We use geometric morphometrics to quantify shape variation for the mandibular, lateral, and ventral craniofacial skeleton from larval into juvenile stages. We find that, despite drastic differences in adult craniofacial morphologies, there is a qualitatively similar path of ontogeny, suggesting that natural selection is working within a conserved developmental program. However, we also detect species-specific differences in the timing, direction, and/or

duration of trajectories, including evidence of heterchrony. Previous work suggests that species-specific differences in adult morphology are due to changes in molecular signaling that regulate early development. In support of this, we demonstrate that modulation of Wnt signaling at early stages can shift a developmental trajectory into morphospace normally occupied by another species. However, without sustained modulation, craniofacial shape can recover by juvenile stages. Overall, this work demonstrates the dual role of development in promoting and constraining phenotypic variation, and underscores that complex morphologies are robust and a compilation of variation in multiple molecular and developmental pathways, acting over extended periods of time. This work supported by NIH/NIDCR 1F32DE023707.

P126 Beck Powers, University of Vermont

Genetic building blocks in the evolution of sympetaly

With over 350,000 documented species, flowering plants (angiosperms) represent the most diverse lineage of vascular land plants on earth. The evolution and subsequent diversification of flowers is posited as a key innovation driving angiosperm speciation, with major modifications on the floral ground plan being linked to shifts in pollinators and reproductive isolation. A dramatic example of this type of floral modification is the evolution of fused petals (sympetaly) to form a corolla tube at the base of the megadiverse Asteridae lineage. Conventionally, the evolution of sympetaly is explained in terms of selection by external factors, most notably, pollinator attraction. However, morphological evolution is also constrained by internal factors, including genetic architecture, phylogenetic history, and development. Here, we seek to elucidate how the ancestral petal developmental genetic pathway has been rewired/elaborated on to affect the evolution of sympetaly in the trumpet-flowered asterid species Petunia x hybrida (petunia, Solanaceae). We hypothesize that changes in the expression and/or function of the organ boundary and lateral expansion genes HANABA TARANU (HAN) and WUSCHEL-like homeobOX 3 (WOX3), respectively, are implicated in the evolution of petunia sympetaly. Both of these genes are expressed broadly in vegetative and floral tissues of petunia, including petals. Furthermore, virus-induced silencing of HAN tentatively suggests that this gene is critical for general growth and development, since HAN-silenced plants die prematurely. We are currently following up on these experiments in order to determine the specific impact, if any, of HAN- and WOX3- silencing on organ fusion within the flower.

P127 Vivek Prakash, Stanford University

Fast and Continuous Epithelial Morphogenesis in a Basal Metazoan

Epithelia are robust tissues that provide support to organs and embryos, and serve as a robust barrier against the external environment. However, epithelia is also known to be a dynamic structure and is capable of morphogenesis and wound healing and self-repair via dynamic rearrangement. Large shape change and cellular rearrangements occur during morphogenesis (intercalation) as the

tissue self-deforms into a new shape. In order to satisfy these two contradictory functions, paradoxically the epithelia is both robust and a continuous solid (avoids any gaps) and a fluid at the same time. To explore the evolutionary context of cellular rearrangements, here we report fast and continuous epithelial morphogenesis in an adult, basal animal - the Trichoplax adhaerens (Phylum Placozoa). By developing in-toto imaging techniques and live microscopy; we demonstrate that T. adhaerens exhibits real-time continuous shape deformation at the whole-organism scale. These shape changes occur due to fluid-like characteristics of a primitive epithelium. Here we demonstrate a new technique to study large scale tissue deformation by binding fluorescent beads to specific cells and single particle/cell tracking over long periods of time. We demonstrate that the cells exhibit non-affine (non-uniform) motion over the timescale of a few seconds. This non-affine motion demonstrates real-time irreversible plastic tissue deformation involving local cellular rearrangements and cellular junction remodeling; which is at the heart of an organismic body plan with no anterior-posterior or radial symmetry breaking.

P128 Meredith Protas, Dominican University of California
Analysis of an integrated transcriptome of cave, surface, and hybrid isopod crustaceans of the species *Asellus aquaticus*
Cave animals are fascinating creatures with interesting morphological characteristics including eye loss, pigment loss, longer appendages, and enhanced sensory systems. Cave animals are excellent species in which to study the genetic and developmental basis of morphological change. Asellus aquaticus is an isopod crustacean with cave and surface dwelling populations. Advantages of this species include the extreme morphological differences between cave and surface populations, the diversity of phenotypes even within a single cave population, and ability to set up crosses between the two morphotypes. We performed 454 sequencing of non-normalized cDNA libraries from four samples, an adult head of a cave individual, an adult head of a surface individual, an adult head of a hybrid individual (crossed between a cave and a surface individual), and a pooled sample of surface embryos and hatchlings. We generated an integrated transcriptome, compiling the reads from all four transcriptomes, with a total of 23,984 contigs. We used this information to isolate many SNPs, place additional candidate genes on our existing linkage map, and to look at allele-specific expression in the hybrid individual. These resources will help to discover genes and genetic changes responsible for cave-specific characteristics and, in tandem with similar studies on other cave dwelling species, will give us a better understanding of the evolutionary processes of cave animals.

P129 Honghu Quan, University of Illinois at Chicago
The evolution of insect germline specification
Germline cells are unique as they can produce gametes and regenerate themselves. Among animals, germ cells can be specified by either maternally inherited determinants or by inductive signals. Among the invertebrates, the only arthropod in which the germ line has been

studied in detail is Drosophila melanogaster. However, this mechanism of germ cell specification is not widespread among, or representative of, all arthropods. Nasonia, like Drosophila, using the maternal inheritance mode to establish its germline, represents an earlier lineage than Drosophila does in Holometabla, which might give the hint to explain the origin of this mode. Tribolium castaneum is an insect model we use to study the mechanism of signal-induced germline specification. The third insect is Callosobruchus maculatus, which shares the same mode of germline specification with Drosophila and Nasonia, but belongs to the order of coleoptera like Tribolium. This might help us understand how the mechanisms evolve over the course of evolution.To understand the conserved and divergent features between Nasonia and Drosophila from the molecular level, one of the most abundant and important components of germ plasm, mRNA, was characterized by RNAseq. We also studied how germ plasm is assembled in the context of teleotrophic oogenesis in Callosobruchus. Oskar gene is considered as sitting on the top of the hierarchy of germ plasm assembly, knocking oskar gene into the genome of Tribolium by CRISPR/Cas9 system will give us insight of the relationship between the two modes of germline specification.

P130 Erik Ragsdale, Indiana University

Forward genetics to reveal the developmental pathway for a morphological polyphenism

The ability to execute different developmental trajectories from a single genotype – namely, polyphenism – allows an immediate, adaptive response to the environment without the need for genetic change. Although many examples of this remarkable phenomenon exist in nature, the functional genetic bases of polyphenism have been mostly elusive. The identification of a developmental switch gene for one case of polyphenism, a feeding-structure dimorphism in the nematode *Pristionchus pacificus*, has provided an inroad to discovering the genetic factors underlying a polyphenism and how these factors and their interactions change to tailor developmental switches to the environment. The switch gene, which encodes the sulfatase EUD-1, promotes the formation of a toothed, predatory ("eurystomatous") morph in response to crowding and deprivation of bacterial food. To test whether a genetic pathway could be known for the *P. pacificus* polyphenism, we used forward genetics to search for downstream targets of EUD-1. Specifically, we conducted a genetic screen for suppressors of a *eud-1* null mutant, which promote the eurystomatous morph in a background completely defective for that morph. As a result, we successfully isolated nine mutant alleles in at least three complementation groups from a screen of 10,300 genomes. The presence of several switch-altering mutants, including recessive and dominant, autosomal and X-linked alleles, affirms the feasibility of reconstructing a switch mechanism for a polyphenism using this model system.

P131 Ana Ramos, Ecole Normale Superieure de Lyon

Exploring the development and evolution of the crustacean eye

The wide diversity of eyes present in arthropods makes them a unique group to study eye evolution. However, most of our knowledge on the

development and neural architecture of the visual system comes from just a few model organisms. Parhyale has an apposition eye, different from the one found in Drosophila (superposition eye). They differ in their optical arrangement but also in the neural architecture of the optic lobe. While it is believed that apposition eyes represent an ancestral state, from which superposition eyes evolved, how did this happen is not understood. To better understand the evolution of the arthropod visual system I am characterizing the cellular composition and neuro anatomy of Parhyale visual system, using new eye transgenic markers. I am also developing a Brainbow-like stochastic cell labeling which will allow a more detailed study of photoreceptor projections into the optic lobe. With this tool it will be possible to know how different projections connect with each other and with the brain and gain insights into the processing of visual signals. Unlike Drosophila, the eye of Parhyale does not have a fixed number of ommatidia. Instead the eye keeps growing throughout life time, raising questions on how/where new photoreceptors are formed and how the fully developed brain copes with the addition of these new cells. The differences between Drosophila and Parhyale eye morphology, also reveals a different mechanism for light intensity adaptation (pupillary mechanism). While in Drosophila the pigment granules responsible for pupil closure migrate in the apico-basal axis of the photoreceptor cell, in Parhyale this migration occurs along the proximal-distal axis. By exploring the tools available in Parhyale, I'm characterizing this movement to elucidate its molecular/cellular mechanism.

P132 Ryan Range, Mississippi State University

Wnt, neuroectoderm, patterning, GRNs

Studies in several deuterostome developmental model systems, including sea urchins and hemichordate embryos, suggest that an early, broad regulatory state initiates specification of the presumptive anterior neuroectoderm (ANE). During development, a posterior-to-anterior wave of inductive signaling progressively restricts this broad ANE potential to the anterior pole, where it is subsequently patterned to form various neural tissues. However, the molecular mechanisms used to position and pattern the ANE along the anterior-posterior (AP) axis are incompletely understood. Our recent studies in the sea urchin show that confining the ANE to the anterior pole involves the integration of information from the Wnt/β-catenin, Wnt/JNK, and Wnt/PKC pathways. We have also found that secreted Wnt signaling modulators synthesized at the anterior pole act as a negative feedback signaling center that establishes the ANE boundary and subsequently patterns the ANE territory. Using a combination of molecular manipulations, high-throughput genome-wide assays, gene regulatory network analysis and classical experimental embryology approaches, we are attempting to produce a systems-level model of these fundamental developmental processes. Recently, we have expanded our functional analysis to the hemichordate, Saccoglossus kowalevskii. Our preliminary data suggest that aspects of the early Wnt network may be shared by ambulacrarians. The phylogenetic position of sea urchins and hemichordates at the base of the deuterostome lineage makes them an

attractive model for studying the evolution of ANE positioning and patterning mechanisms in deuterostomes.

P133 Gustavo Rezende, Universidade Estadual do Norte Fluminense
Evolution of desiccation resistance in eggs of mosquito vectors are related to eggshell attributes
Mosquitoes are vectors of parasites that cause diseases such as malaria and dengue. Mosquito eggs are laid in water and are susceptible to dehydration, a condition that can impair their viability. During embryogenesis of Aedes aegypti, the dengue vector, the extraembryonic serosa surrounds the embryo and secretes the serosal cuticle, fundamental for the acquisition of egg resistance to desiccation (ERD), enabling developing embryos to live in dry environments. Conservation of both serosal cuticle presence and ERD acquisition throughout mosquito evolution is unknown. The process of EDR acquisition was evaluated for Aedes aegypti, Anopheles aquasalis and Culex quinquefasciatus, belonging to different genera, exhibiting distinct evolutionary histories and habits. The total embryogenesis period varies among species; ERD is acquired after serosal cuticle formation but at distinct embryonic stages. Viability varies in dry conditions: Aedes, Anopheles and Culex eggs can survive, respectively, for 72, 24 and 5 hours (high, medium and low ERD). Various egg features were then evaluated: length, width, area, volume, weight, superficial density, chitin presence and content and endochorion external surface aspect. Although interesting differences were observed for all aspects, only three could be related with ERD levels: volume increase during embryogenesis and eggshell superficial density are inversely related with ERD while chitin content is directly related with ERD. These results suggest that eggshell chitin amount is relevant for a more efficient ERD but that other yet unidentified egg attributes must also be considered in order to account for the distinct levels of ERD observed among these three species.

P134 Ajna Rivera, University of the Pacific
The Pax/Six Gene Regulatory Network in a demosponge, Ephydatia muelleri
The evolution of gene regulatory networks is the crux to understanding how genetic changes can spur morphological evolution, in particular the evolution of novel complex features. Demosponges, like Ephydatia muelleri, are an exciting new model for elucidating the evolution of gene regulatory networks as they have pared-down genomes compared to other model systems. Previously, we showed that the “retinal determination” genes Pax and Six interact in E. muelleri using RNAi knockdown, qPCR, EMSA, and ChIP assays. Here we expand this gene regulatory network by finding additional sponge targets of the Pax transcription factor. We used a bioinformatics approach to find potential Pax targets in the E. muelleri genome and then tested these using dual luciferase assays in mammalian cell culture. In this way, we aim to develop a more high-throughput method of discovering components of a metazoan-specific gene regulatory network in a simple animal.

P135 Wade Roberts, Washington State University

Understanding Flower Diversification in *Achimenes* (Gesneriaceae) Using a Comparative Transcriptomics Approach

Achimenes is a genus of 26 species in the Gesneriaceae, native to Mexico and Central America, which shows remarkable variation in floral form. This includes flowers that are funnelform, salverform, or tubular, white, yellow, pink, purple, and red, and with or without corolla spurs. Floral form appears to be quite variable among closely related species with homoplastic derivations of shape, color, and corolla spurs. As such, this genus represents a unique opportunity to study the molecular genetic mechanisms involved in the development and diversification of flowers in *Achimenes*. In a comparative study, we performed RNA-seq across four species and three developmental time points in order to examine gene expression differences related to the floral phenotypes. Samples were taken from immature bud, stage D, and pre-anthesis flowers in the following species: *A. cettoana* (purple, salverform), *A. erecta* (red, salverform), *A. misera* (white, funnelform), and *A. patens* (purple, salverform, corolla spur). Transcriptomes were assembled utilizing multiple *de novo* assembly programs and merged to produce a final contig assembly containing primary and secondary transcripts. Reads pertaining to developmental stages were mapped back to the contigs and estimations of relative gene expression were produced. We identified large sets of co-expressed genes by clustering with a Poisson mixture model and will predict key biological processes that are differentially enriched in each cluster. Further characterization will involve orthology assignment and detailed annotation. This study provides novel biological insights into the molecular mechanisms involved in flower diversification in *Achimenes*.

P136 Rachel Roston, Duke University

The Ontogeny of Telescoping in Cetaceans

Among their many secondary modifications for obligate aquatic life, cetaceans (whales, dolphins, porpoises) have highly divergent skulls that seem to defy typical mammalian skull structure. In the course of the evolution of cetacean skulls, a process known as telescoping, the rostrum has elongated, the nares have become dorsally positioned, and skull bones have come to overlap extensively. In this project we compare members of the two extant clades of cetaceans, examining various stages of skull ontogeny in relation to absolute time, gestation period, and body size. Rostral elongation begins early in development and the overlapping of skull bones develops later, during the fetal period. In addition, during fetal period, baleen whales (mysticetes) experience the highest growth rates of any mammal and attain large body sizes at birth without correspondingly long gestation periods. In contrast, toothed whales (odontocetes) are much smaller at birth and their gestation periods vary with birth size. While cetacean fetuses are relatively rare in museum collections, non-invasive imaging has made data from them more accessible for comparative studies. Using an invaluable resource of CT scans of cetacean fetuses in the NMNH collection, we document and compare the ontogeny of cetacean skulls in multiple species in the context of their overall fetal growth.

P137 Alexa Sadier, University of Illinois at Urbana-Champaign

Tinkering signaling pathways by gain and loss of protein isoforms: The case of the EDA pathway regulator EDARADD

Background- We highlight a role for alternative splicing/promoter initiation in signaling pathways diversification by taking the example of the two A and B isoforms of EDARADD, the intracellular adaptor of the Ectodysplasin pathway which is known to be implicated in Vertebrate evolution. Results- We obtained sequences of the gained A isoform and the B isoform in several mammals representative of the phylogeny and showed that the mammal-specific A isoform is repeatedly lost in terminal lineages throughout mammal phylogeny whereas the isoform B is conserved. To investigate the functional consequences of these gain/losses, we then characterized the respective functions of the two isoforms in cellulo and in vivo. We first showed that the two isoforms are transcribed from two conserved alternative promoters that exhibited feedback regulation. We then studied their expression in a various set of tissues and cell lines and found that they are frequently co-expressed, but with a tissue-specific regulation of their respective expression level. We then showed that both isoforms activate the NF-κB pathway, albeit at different levels and dynamics since the A isoform was downregulated following pathway activation. Finally, we found that only the B isoform could rescue a zebrafish edaradd knockdown. Conclusions- We concluded that having two isoforms enable a fine context-specific regulation of the EDA pathway, which evolved rapidly during mammal diversification. We further propose that alternatively spliced isoforms with close function but divergent regulation could play a driving role in phenotypic evolution by ensuring that essential roles are maintained while offering an evolutionary plasticity.

P138 Leyli Samadi, University of Vienna

Molecular basis of eye development in the spider Cupiennius salei

Gene-transcription factors that determine retinal development seem to be conserved in different phyla throughout the animal kingdom. In most representatives, however, only a few of the involved transcription factors have been sampled and many animal groups remain understudied. In order to fill in the gaps for the chelicerate group of arthropods, we tested the expression pattern of the candidate genes involved in the eye development in the embryo of the wandering spider Cupiennius salei. We tested the expression of dpp, hedgehog, glass, ndf and wingless in the spider embryos. The genes are mainly expressed in the developing optic neuropiles of the eyes (lateral furrow, mushroom body and arcuate body) in earlier stages of development (160-220h after egg laying). Later in the development (180-280h after egg laying), there is differential expression of the genes in disparate eye vesicles. Our data elucidate that the genes involved in the eye development in other metazoans are conserved in spiders however the genes are deployed differentially to differentiate each particular eye type of the spiders.

P139 Andres Sarrazin, Pontificia Universidad Católica de Valparaíso
Signaling pathways involved in *Tribolium castaneum* gene oscillation-dependent germband elongation
Most arthropods generate the posterior part of their bodies by adding segments sequentially from the rear part of the embryo (growth zone, GZ), just like vertebrate somites arise from presomitic mesoderm. Remarkable conserved similarities have been found in the molecular mechanisms involved in the generation of a segmented body plan by these two phyla. In vertebrates, axis elongation and segmentation are connected by the Wnt and FGF signaling pathways. There is also evidence that during arthropod development, Wnt signaling participates in axis formation and GZ establishment at the posterior end of the embryo. Since some Wnt family genes continue expressing in the GZ, we are interested to know their role after the onset of germband elongation. In order to gain access to this process, we developed a whole embryo culture approach to test different signaling pathways inhibitors/activators during specific time intervals, in such a way to determine changes in the segmentation period, elongation rate and wavefront establishment. Ectopic activation of Wnt signaling by LiCl incubation, a GSK3 inhibitor, produced shorter germbands compared to the control. Additionally, the small molecule IWP-3, that inhibits Wnt signaling by blocking Wnt ligands secretion, gave rise to longer germbands. When we analyzed the expression pattern of the genes odd- and even-skipped, we found that Wnt pathway activation and inhibition of FGF signaling by SU5402, both abolished the expression of these cyclic genes in the GZ, suggesting both, Wnt and FGF signaling pathways are involved in the regulation of the oscillation-dependent Tribolium germband elongation.

P140 Aditya Saxena, University of California, San Diego
Genetic Control of Limb Elongation In Lesser Egyptian Jerboa
Tremendous diversity exists in the lengths and sizes of mammalian long bones. However, the genetic mechanisms that generate our long arm bones and short finger bones, for example, remain unclear. We use the Lesser Egyptian Jerboa, a close relative of the laboratory mouse, as a model to understand the genetic and developmental basis of limb bone elongation and allometric scaling of proportion. The jerboa is a bipedal rodent that has extraordinarily elongated hindlimbs, particularly the metatarsals, compared to mice. Limb bone elongation is largely controlled by the extent of cell volume increase of post-mitotic hypertrophic chondrocytes in the growth plate. Rapidly elongating bones, such as the jerboa metatarsal, have larger hypertrophic chondrocytes than the homologous mouse metatarsals. In this study, we extend our previous observation that the amount of cellular mass produced after cytoplasmic fluid swelling determines the difference in cell size and thus bone growth rate. We employ an RNASeq approach on metatarsal cartilage cells to uncover the genes that are differentially expressed in the jerboa and mouse metatarsals that differ in growth rate compared to the ulna that elongates at a similar rate in both species. Results of this investigation will pinpoint genes that play a putative role in allometric scaling of the skeleton. Future experiments will focus on

gene regulatory elements at these loci to determine the genetic mechanisms that shape organisms by differential deployment of a conserved set of skeletal development genes.

P141 Klaske Schippers, University of Denver

Using sponges as a model to examine the evolution of the Wnt/beta-catenin signaling pathway

The discovery of conserved homologs of the Wnt/beta-catenin pathway in sponges (one of the earliest branching metazoan lineages) raised questions about whether a functional Wnt/beta-catenin pathway is present in sponges and what its role may be in organisms of such relative simplicity. To gain more insight in the role of beta-catenin in sponges, we identified tissue-specific and subcellular localization patterns of beta-catenin by performing immunostaining using a polyclonal antibody against beta-catenin of the freshwater sponge Ephydatia muelleri. Our immunostaining data show that beta-catenin is detected in the nuclei of archeaocytes (i.e. mesenchymal cells) and pinacoderm cells (i.e. epithelial cells), suggesting a conserved role as a transcription factor, possibly part of the Wnt pathway. We also observed staining at cell boundaries of the pinacoderm, which is consistent with a role in cell-cell adhesion. Staining was not detected in cell boundaries of the choanoderm (composed of choanoflagellate-like cells, that pumps water through a water canal system used for feeding and respiration), which could indicate this tissue does not use cadherin/catenin adhesion mechanisms.

To further test the role of beta-catenin in sponges, we will use Co-IP to identify binding partners of beta-catenin; Chip-sequencing to identify target genes that are regulated by the beta-catenin/TCF transcriptional complex; and develop techniques for studying gene function in vivo in sponges, so we can silence or overexpress beta-catenin in vivo.

P142 Patricia Schneider, Universidade Federal do Para

Ontogeny and eye RNA-seq analysis reveal visual and non visual opsin repertoire of the four-eyed fish (*Anableps anableps*)

The evolution and development of the eye has intrigued developmental biologists for centuries. Aside from partial or complete loss, few vertebrates display substantial modifications to the eye morphology. The four-eyed fish Anableps anableps consists in a unique model system to study eye Evo-Devo due to its distinctive feature of having duplicated eye structures. This species is commonly found in the amazon region and reproduces throughout the year. Featuring “split eye“ this species is capable of looking above and under the water level and has duplicated structures such as pupils and cornea and a functionally duplicated retina. The retina is divided into dorsal and ventral regions and photoreceptors have been shown differential expression pattern in the adult fish. Our goal is to describe these larval stages and to characterize morphological and molecularly the retina during eye development. We have identified at least seven distinct developmental stages for A. anableps. Furthermore, our transcriptome analysis has identified visual and non-visual opsins expressed in developing eyes. We find asymmetric opsin protein expression in the

developing retina. The result of this study will shed light on the molecular basis of this innovative feature.

P143 Richard Schneider, University of California, San Francisco
Developmental mechanisms regulating jaw size evolution
The remarkable success of vertebrates is due in large part to the ability of the jaw to vary in size during evolution. A wide range of genetic and embryological studies have shown that the establishment of jaw size is a complex process involving numerous gene regulatory networks, reciprocal signaling interactions, and hierarchical levels of control. Yet what remains unclear are precise determinants of species-specific jaw size that presumably operate during the allocation, proliferation, differentiation, and growth of jaw progenitors. To address this issue, we use Japanese quail, which have short jaws, and white Pekin duck, which have relatively long jaws. We transplant neural crest mesenchyme (NCM), which are the progenitor cells destined to form the jaw skeleton, between quail and duck embryos. Resulting chimeras are challenged to integrate two distinct morphogenetic programs and enable us to pinpoint mechanisms underlying the establishment of species-specific jaw size. We have found that NCM employs a variety of precise mechanisms to govern jaw size through three principal phases of development. First, during the migration and allocation of NCM, quail and duck have distinct numbers of progenitors destined to form the jaw skeleton; second, when these populations proliferate and expand, there is species-specific regulation of, and response to, multiple signaling pathways; and third, as these progenitors differentiate into the jaw skeleton, they execute autonomous molecular and cellular programs for bone deposition and resorption intrinsic to each species. Thus, NCM deploy sequential but distinct molecular and cellular mechanisms throughout development that regulate jaw size during evolution.

P144 Jennifer Schwab, Mississippi State University
Sexually Dimorphic Plasticity on the Wings of the Dogface Butterfly
The vast array of biodiversity and natural variation that we see around us has been generated through a combination of genetic and environmental influences. Traits that are environmentally induced within species, but are genetically different between species provide a framework for identifying the developmental pathways that drive the evolutionary process. Butterfly wing patterns provide an amenable evolutionary model to study these genetic and environmental interactions. We aim to use the Dogface butterfly, Zerene cesonia, to study these processes. Z. cesonia is a seasonally polyphenetic butterfly varying in its pterin pigmentation, having a yellow summer form and a pink winter form. These environmentally induced pterin differences within species also reflect the pterin differences expressed between Z. cesonia and its sister species the California Dogface, Zerene eurydice. Currently, we are characterizing the developmental plasticity within species to better understand the environmental drivers of developmental plasticity. Conditional rearing under summer and winter conditions were conducted to successfully reproduce both seasonal

forms. Unexpectedly, sexual dimorphism in the winter form expression was observed. Females expressed the winter form at a warmer temperature threshold than males. Also males, who normally have ultraviolet coloration on their hindwings, lost hindwing UV expression in the winter form. Our results show sex specific regulation during the development of the seasonal form in Z. cesonia as well as a possible connection between pigment, pterin, and structural, UV, coloration determination.

P145 Mark Seeger, Ohio State University

Commissureless regulation of Slit-Robo signalling in insects

Slit-Robo signaling is a key mediator of axon guidance decisions in divergent organisms ranging from planaria to vertebrates. Not surprisingly, Slit and Robo homologues can be identified in all of the sequenced insect genomes. In contrast to this conservation of ligand and receptor, organisms have evolved various mechanisms to regulate Slit-Robo signaling. In Drosophila, Commissureless is a key post-translational regulator of the Robo receptor that functions to prevent cell surface accumulation of Robo. Two additional Comm-family members are found in Drosophila and they vary in their ability to regulate Robo receptors. We are investigating the evolution and function of Comm-like genes in insects. Bioinformatic studies indicate that Comm-like genes are present in most Dipteran genomes, although the number of Comm-family members varies. Divergent Comm-like genes can be identified in representatives of Trichoptera, Coleoptera, Hymenoptera, Phthiraptera, Hemiptera, Blattaria, Ephemeroptera, and Odonata, but not outside of insects. The presence of a Comm-like gene in many diverse insect orders suggests it was present early in insect evolution. There is evidence supporting three independent losses of this Comm-like gene: 1) the absence from sequenced Lepidopteran genomes, 2) the absence from Tribolium but presence in more basal Coleopteran genomes, and 3) the presence in basal Hymenoptera, like the sawfly, and absence in more derived Hymenoptera including ants, bees, and most wasps. In ongoing experiments, we are addressing the functional properties of divergent Comm-family members from a variety of insects using several approaches, including RNAi and a Drosophila cell culture assay for Robo regulation.

P146 Bharti Sharma, California State Polytechnic University, Pomona

Unraveling new players in the control of inflorescence structure in *Aquilegia* (columbine)

The transition to flowering involves complex interactions between the genetic programs controlling inflorescence and floral meristem identity, which result in a wide variety of inflorescence structures. From previous studies in model systems including Arabidopsis, tomato and Petunia, it has been establish that LEAFY and its coMfactor UFO are involved in establishing floral meristem identity (FMI) and subsequently activating the floral organ identity genes, including most of the players in the ABC model. We have used the lower eudicot model Aquilegia to investigate the conservation of LFY and UFO homolog function, in both of these developmental contexts. In Aquilegia there are two UFO paralogs,

AqUFO1 and AqUFO2, and a single copy of7AqLFY. The expression of these genes is consistent with the patterns observed in core eudicots, with AqLFY expressed throughout early floral meristems while AqUFO1 is tightly restricted to the base of the initiating petals. AqUFO2, in contrast, is primarily expressed in the distal tips of the stamens. Using the transient RNAi method virus induced gene silencing, we downMregulated AqLFY as well as AqUFO1 and AqUFO2 individually and in combination. The phenotypes obtained from AqLFYMsilencing indicate a less essential role in floral meristem identity, although an increased degree of branching suggests that AqLFY does contribute to inflorescence structure. Interestingly, the AqUFO18 and AqUFO2Msilenced phenotypes were more severe than that of AqLFY. In addition to excessive branching and other weak floral meristem identity defects, we observed a complete loss of petal identity. This suggests that the Aquilegia UFO homologs play a specific role in activating a petalMspecific paralog of the B class gene APETALA3. We will discuss these functions and their implications for the evolution of the floral meristem identity network in Aquilegia.

P147 Jen Spengler, Millersville University

Trunk neural crest cell specification and emigration in turtle embryos

Turtle plastron bones develop by intramembranous ossification, suggesting that they are derived, like the facial bones, from neural crest cells. Using cell-labeling and neural tube explant cultures, we have shown that cells expressing neural crest markers emerge from the trunk neural tube in the turtle Trachemys scripta for a greatly extended period, well beyond the stage of neural crest emigration in chick or mouse embryos. The neural crest cells that emerge late appear to migrate ventrally to form an ectomesenchymal dermis that gives rise to the bones of the plastron. These late emerging neural crest cells also express PDGFRá, typically expressed only by cranial neural crest cells. In addition, late emerging cells coalesce in culture into nodules expressing markers of osteoblast differentiation, suggesting their role in plastron bone formation. Although there appears to be two distinct migratory phases in vivo, neural crest cells can be observed in vitro emigrating from neural tubes isolated from embryos, between the periods of in vivo migration. We are currently examining the expression of markers of premigratory and early migratory neural crest cells to examine whether the premigratory domain persists during the period between the early and late migratory phases. If this is the case, it would suggest the lack of neural crest cell migration may be due to the lack of a supportive environment in the embryo. In addition, we are examining the gene expression profile and differentiation potential of neural crest cells isolated from embryos throughout the window of neural crest cell migration.

P148 Tyler Square, University of Colorado Boulder

The role of the endothelin pathway in vertebrate evolution

The head skeleton represents a key developmental innovation which immediately precedes adaptive radiation at the base of the vertebrate

clade. Although cellular cartilage itself appears to predate vertebrates, a *bona fide* head skeleton evolved in stem vertebrates by spreading and shaping this tissue throughout the head. The endothelin pathway has been shown to have particular effects on morphogenesis in the craniofacial skeleton of zebrafish and mouse; here we explore the expression and functional significance of endothelin pathway genes in the sea lamprey (*Petromyzon marinus*) and the African clawed frog (*Xenopus laevis*). We find that the sum of endothelin receptor expression is well conserved in space and time in both of these vertebrates, however lamprey and gnathostomes seem to have subfunctionalized their receptors in quite different ways both spatially and temporally. Endothelin ligand expression tends to be less conserved; some of these obvious differences correlate well with the stronger, directional pharyngeal arch polarity seen in gnathostomes as compared to lamprey. We also present some preliminary data from functional perturbations on these genes using CRISPR/Cas9 mediated mutagenesis.

P149 Thomas Stewart, University of Chicago

The development of adipose fins

Adipose fins are appendages found on over six thousand species of actinopterygian (ray-finned) fishes. Although historically regarded as non-functional and vestigial, recent analyses have found that adipose fins are novelties that evolved repeatedly in teleost fishes. To inform hypotheses of adipose fin origin and function, I characterized the ontogeny of the adipose fin of Corydoras aeneus (Callichthyidae), a South American armored catfish. In C. aeneus, adipose fin development involves the retention and elaboration of a larval structure, the larval fin fold. During adipose fin development, a condensation forms halfway along the proximo-distal axis of the larval fin fold. This condensation ossifies to become the adipose fin spine. The larval fin fold reduces anterior and posterior to the adipose fin domain and the fin spine extends both proximally and distally until it reaches the body wall; at this point, adipose fin nerves are observed. These nerves appear to originate from dorsal rami of the spinal cord and from the recurrent ramus of the facial nerve. The primary adipose fin nerve runs along the proximo-distal axis of the fin posterior to the fin spine, ramifying repeatedly and extending projections posteriorly into the fin membrane to terminate as free nerve endings and taste buds. I conclude by discussing how these data constrain hypotheses of adipose fin origin, inform the hypothesis that adipose fins function as mechanosensors, and highlight challenges to applying novelty concepts to structures with apparent serial homologs.

P150 Harold Suárez-Baron, University of Antioquia

Flower development and perianth identity candidate genes in the basal angiosperm *Aristolochia fimbriata* (Piperales: Aristolochiaceae)

Aristolochia fimbriata is a member of an early diverging lineage of flowering plants and a promising candidate for evo-devo studies. Aristolochia flowers exhibit a unique floral synorganization that consists

of a petaloid sepal-derived perianth, no petals, and a congenital fusion of stamens and carpels into a gynostemium. We investigated the floral development and morphology of A. fimbriata, and evaluated the expression of key regulatory MADS-box genes that are likely responsible for the identity of perianth and gynostemium. MADS-box ABCDE gene homologs were isolated and were included in phylogenetic analyses to confirm their placement as pre-duplication genes prior to the γ polyploidization event occurring in core-eudicots. Additionally, RT-PCR and in situ hybridization were performed to identify gene expression patterns and hypothesize gene functions. AfimFUL and AfimAG show broad expression patterns in all floral organs leaves and fruits, contrary to the canonical mutually exclusive expression patterns shown by the orthologs (A and C class genes) in the model eudicot Arabidopsis thaliana in sterile and fertile floral whorls, respectively. .B-class genes, AfimAP3 and AfimPI are broadly expressed early on throughout the flower but become restricted to the distal portion of the perianth at anthesis. The D class gene AfimSTK is only turned on in the gynostemium and the ovary. Finally, AfimAGL6 shows a restricted expression pattern to the perianth. Our results suggest that the ABCDE model can be partially extrapolated to early diverging angiosperms and postulates AGL6 as a candidate gene for sepal identity in Aristolochia.

P151 Vanessa Suaza-Gaviria, University of Antioquia

A comparative developmental study of inflorenscences and flowers in hemiparasitic plants in the sandal wood order

Parasitic flowering plants possess extraordinary reproductive and dispersal strategies including longer reproductive phases, a vast production of flowers with copious amounts of nectar, sticky fruits or seeds, and rapidly growing embryos with early ability for host penetration. The order Santalales contains the largest group of neotropical hemiparasites. We studied inflorescence and floral development in members of Loranthaceae (Gaiadendron punctatum, Phthirusa stelis, P. pyrifolia and Oryctanthus occidentalis) and Viscaceae (Dendrophthora avenia and Phoradendron nervosum), with the aim of identifying ontogenetic features that have been fixed phylogenetically. All Loranthaceae have branches with indeterminate growth and superior ovaries forming berries. In addition, they form large numbers of dichasia (i.e. inflorescences with a terminal flower accompanied by two lateral flowers) carrying hexamerous bisexual flowers with sepals, colored petals; and often an extra whorl,the epicalyx forms outside of the sepals. Oryctanthus is atypical among Loranthaceae as only the central flower of the dichasium develops embedded into a thickened axis; lateral flowers are aborted. In Viscaceae, a remarkable system of modular inflorescences, likely derived from modified dichasia, occurs; the modules are formed by tiny trimerous, unisexual flowers with a single whorled perianth. The staminate flowers present a vestigial gynoecium, and the carpellate flowers possess a nectarial ring instead of the missing stamens. We provide evidence that inflorescences in Viscaceae are due to syndesmy (i.e. congenital fusion between the central and lateral dichasia with the

primary axis of the inflorescence). Despite major differences in inflorescence architecture, the earliest developmental stages are shared between Viscaceae and Loranthaceae.

P152 Yuichiro Suzuki, Wellesley

The origin of insect larval form

One of the biggest mysteries in insect evolution is the origin of complete metamorphosis in insects. For over a century, the origin of larval morphology has been debated, but consensus has not been reached. Much of the evidence presented to date have focused on gross anatomical comparisons or endocrine regulation, which may evolve rather quickly. Instead, examining the origins of imaginal cells, set-aside cells that ultimately give rise to the adult form, may hold the key to understanding how the larval form evolved because these cells are only found in insects undergoing complete metamorphosis. Here, the role of Hedgehog (Hh) signaling in the development of imaginal cells was examined in the flour beetle, Tribolium castaneum. RNA interference-mediated knockdown of hh showed that Hh is required for the proliferation of imaginal cells. In contrast, knockdown of Hh signaling antagonists led to the overgrowth and precocious maturation of structures derived from imaginal cells and the occasional appearance of ectopic appendages from the head epidermis. Our findings, together with our work on Hh signaling in a hemimetabolous insect, suggest that Hh signaling played a critical role in the evolution of imaginal cells. A model will be presented to explain the origin of the larval form.

P153 Billie Swalla, University of Washington

A Tale of Molgulid Tails and Developmental Heterochronies in Ascidians

Transcriptome and genome data offer new approaches to examine the origin and evolution of the chordate body plan. Chordate body plan evolution has been studied by comparing two closely related ascidian species with radically different larval body plans — the tailed Molgula oculata and the tailless M. occulta. Embryos of tailed M. oculata have 40 notochord cells that converge and extend to form the notochord in the center of the tadpole larva's tail, like most ascidians. Muscle cells flank the notochord in the tail of M. oculata and are critical for larval swimming. In the head is the otolith, a gravity-sensing organ that is important for larval settlement at metamorphosis. In contrast, the larva of M. occulta does not have a tail. The embryo has only 20 notochord cells, and these cells do not converge and extend during larval development, but they do form a "notoball". We show by transcriptome analyses that the ascidian metamorphosis program begins earlier in molgulid ascidians than in other ascidian species. This heterochronic shift has been documented in another tailless ascidian, Molgula tectiformis, and is now reported for both the tailed, Molgula oculata and tailless Molgula occulta. Further functional data will be needed to test the hypothesis that this pronounced heterochrony is a preadaptation for evolution of tailless development in molgulid ascidians. These studies will also facilitate the identification of the genes involved in initiating metamorphosis in ascidian tadpole larvae.

P154 Jennifer Tenlen, Seattle Pacific University

Techniques and Resources for the Molecular, Cell Biological and Genetic Analysis of Tardigrades

We have developed a number of techniques and resources for studying tardigrades. In an effort to make these techniques and resources more accessible and to advance tardigrade research, we have made them publically available. Here we present an overview of our latest developments as well as how researchers can access our protocols and other resources. These techniques include: molecular biology (large-scale extraction of nucleic acids and proteins, performing PCR and RT-PCR on single tardigrades, and RNA interference for targeted disruption of tardigrade gene function); cell biology (protocols for immune-labeling, fluorescence staining, and alkaline phosphatase staining), and techniques for culturing and and long-term cryopreservation of the tardigrade *Hypsibius dujardini*. We will also discuss the application of these techniques to tardigrade research in a primarily-undergraduate institution.

P155 Matthew Terry, University of Texas, Pan American

Origin and Evolution of the Insect Body-Plan: Using transcriptomes to expand our view beyond beetles and flies

Insects are the most biodiverse group of organisms on earth and have an instantly recognizable body-plan: a sensory organ rich head, thorax with three pairs of legs, and an abdomen without appendages. Most of what we know about the origin and development of this body-plan is derived from a small number of studied insects. Most data to date comes from groups such as flies, beetles and true bugs; which have a typical insect body-plan, but represent varying amounts of derivation from the ancestral insect form and only represent a small minority of higher level insect diversity. To better understand the evolution of the early insects we need to more broadly sample insects, choosing insects based upon their phylogenetic position and features that represent remnants of abdominal structures lost in most extant insects. Modern transcriptomics, coupled with comparative molecular evolution and gene expression data for critical developmental genes allows us to expand our sampling of insect diversity and shed light on the origin and early evolution of the insects. Analysis of mixed embryo transcriptomes from six species of hexapods [Collembola (2 species), Ephemeroptera, Odonata, Embiidina, Orthoptera) and one crustacean (Artemia) yields complete transcripts for a majority of Hox genes. Hox analyzer, a program developed for post-assembly analysis of transcriptomes, also identifies complete transcripts for many other important developmental genes (Dll, en, Eve, Cad, etc.) Preliminary expression studies are yielding new data bearing on the formation of atypical insect body plans in groups such as Collembola.

P156 Rachel Thayer, University of California, Berkeley

Genetic basis of structural color in the buckeye butterfly

Structural color is caused by constructive interference of light as it interacts with nanoscale, regularly-spaced physical structures on or in

the organism. This is in contrast to pigmentary color, caused by molecules that selectively absorb certain wavelengths. While the genetic basis of pigmentary coloration has been a fruitful research focus from the earliest days of genetics, almost nothing is known of the genetic architecture for structural color. There are a few examples where the inheritance pattern of a structural color is reported (i.e. Uta stansburiana, Colias eurytheme), but no implicated genes or pathways. Nevertheless, structural coloration is employed by many taxa as diverse as humans and Selaginella algae, and can be very adaptive, as when it is used for crypsis or mate attraction. Because the resulting color is strongly dependent on the thickness and regular arrangement of structural elements, structural color is also interesting from a morphogenetic perspective. I am conducting forward genetic mapping of QTL controlling structural coloration in the buckeye butterfly, Junonia coenia. I crossed two lines of buckeyes. One is a well characterized, plain brown line established by Fred Nijhout, for which a transcriptome exists and genome assembly is underway. The second line was artificially selected by butterfly breeder Edith Smith to be prominently covered with blue iridescent scales. Here I present my progress toward describing the genetic architecture of both percent wing coverage and hue of structurally colored scales. I am also using electron microscopy to characterize the responsible nanostructures in this and closely related species.

P157 Gerald Thomsen, Stony Brook University

Exploring the role of candidate stem cell pluripotency genes in sea anemone (Nematostella) regeneration

The Starlet Sea Anemone, Nematostella vectensis, is a great system to explore embryonic developmental mechanisms and their evolution, but this anthozoan cnidarian is also highly regenerative and can renew all of its body from nearly any rudiment. Knowledge of the cellular and molecular mechanisms underlying regeneration in Nematostella, however, is just beginning to emerge. We have been investigating how cut fragments of the adult aboral end (the physa) are capable of regenerating nearly all missing structures. We are attempting to define the cellular and morphological progression of regeneration from physa explants, and by asking whether orthologs of bilaterian pluripotency genes, stem cells, and common developmental signaling pathways, govern regeneration. We have found that the physa exhibit a burst of cell proliferation, post-wound healing, which is accompanied by expression of candidate pluripotency and germline genes (e.g. Cniwi, Klf4/7, Nanos, Pou3/5, SoxB1) as the physa regrows its missing parts. Whether stem cells or other cellular mechanisms (e.g. dedifferentiation-redifferentiation) fuel the physa's regenerative progression is still being investigated. Whatever the outcome, our results should help inform the evolution of regenerative mechanisms among the cnidarian taxa and across the metazoa.

P158 Daniel Urban, University of Illinois at Urbana-Champaign

Mechanisms of Mammalian Middle Ear Ossicle Transition from the Reptilian Jaw Joint

During synapsid evolution, postdentary elements in the reptilian jaw transitioned into the middle ear of early mammals. Separation from the dentary allowed unconstrained evolution of the middle ear ossicles, resulting in increased hearing sensitivity and amplified frequency range. Given the importance of such an innovation, it is essential to understand the evolutionary pathway that led to the current phenotype, as this has implications for understanding the development of all mammals. Using an extant model system, Monodelphis domestica, we investigate the developmental basis of the definitive mammalian middle ear. We utilize micro-CT imaging to characterize the morphological events underpinning the transition, cryosectioning and immunohistochemistry to identify cellular processes behind the morphological events, and laser capture microscopy followed by RNA Sequencing to identify changes in gene expression driving the cellular processes. Among our findings are: decreasing size and rearward movement of the middle ear ossicles appear to be false illusions created by the continued growth and expansion of the surrounding skull elements; separation of Meckel's cartilage from the malleus, occurring at postnatal day 20, is facilitated by apoptosis and is a prerequisite for the onset of hearing; separation and functional changes are due to alterations in timing and expression levels of key genes.

P159 Berta Verd, Centre for Genomic regulation (CRG)

A damped oscillator governs posterior gap gene patterning in *Drosophila melanogaster*

Insects use two main modes of segment determination during development: the ancestral short-germband mode (eg. Gryllus bimaculatus), where new segments are added sequentially, and the long-germband mode (eg. Drosophila melanogaster) where all segments are detemined simultaneously. In dipteran insects (flies, midges and mosquitoes), where the long-germband mode of segmentation is used, the gap genes are activated by maternal gradients and cross regulate each other to form the first zygotic regulatory layer of the segmentation gene hierarchy. A precise mathematical model of the gap genes in Drosophila melanogaster was obtained from quantitative spatio-temporal expression data and used to study the dynamics of pattern formation. This approach showed that two distinct dynamical regimes govern anterior and posterior trunk patterning. Stationary domain boundaries in the anterior rely on bistability. In contrast, the observed anterior shifts of posterior gap gene domains can be explained as an emergent property of an underlying regulatory mechanism implementing a damped oscillator. We have identified a dual-function three-gene motif embedded in the gap gene regulatory network which is sufficient to recover both anterior and posterior dynamical regimes. Which one governs a given region depends on the gap genes involved. This motif is known as the AC/DC circuit. The dynamical repertoire of this motif consists of only one more possible regime, sustained oscillations, which are not found in the gap

gene system. Since molecular oscillations are characteristic of short-germband segmentation, these findings suggest that the two modes of segment determination may have more in common than previously thought. This insight helps us understand why long-germband segmentation may have evolved several dozen times independently from the ancestral short-germband mode.

P160 Pelin Volkan, Duke University

Mechanisms of Development and Evolution of the Olfactory System in Drosophilids

One fascinating aspect of evolutionary history is the rapid diversification and expansion of various different groups of organisms to rapidly fill a host of wildly different niches as they become available. The evolution and diversity of the Drosophila genus is a fine example of this phenomenon. Underlying this diversity is a number of different factors, including the flexibility and rapid evolution of the Drosophila olfactory system that allow the adaptation of different Drosophila species to different environments for foraging and mating. Our hypothesis is that the evolutionary flexibility of the Drosophila olfactory system is provided for by the combinatorial and modular structure of transcription factor networks that govern the specification and expression of olfactory receptor neurons (ORNs) during early development. To investigate this, we have conducted a comparative analysis of the antennal transcriptome of four different Drosophila species at multiple points during development. Our analysis suggests several interesting patterns in the antennal transcriptome between different species, including differences in the transcription factor network governing ORN specification that may support a model in which specific patterns of transcription factor expression creates zones of variability in ORN expression which allows the differential expression of olfactory receptors according to each species' specific ecological needs. Together, these findings may shed light on specific mechanisms by which variability in the olfactory system is produced, and highlight the plasticity of the insect olfactory system in support of the diversity and success of the Drosophila genus.

P161 Tomasz Walski, Gent University

***N*-glycosylation: A new player in insect metamorphosis**

N-glycosylation is a ubiquitous protein modification resulting in the attachment of sugar chain through asparagine. It is estimated that even 30% of all the proteins can be *N*-glycosylated. Despite being vital to a number of phenomena including signaling, immunity and development, *N-glycosylation* received little attention in invertebrates other than *D. melanogaster* or *C. elegans*. The aim of this work was to analyze the involvement of *N*-glycosylation in post-embryonic development of the red flour beetle, *Tribolium castaneum*, a model beetle and important pest insect. To study this subject we analyzed developmental expression patterns of 21 genes involved in protein *N*-glycosylation by qPCR. Secondly, we employed mass spectrometry to compare composition of glycans decorating larval and adult proteins. Finally, we silenced expression of the genes encoding *N*-glycan processing

enzymes using RNAi to examine their physiological roles in metamorphosis. qPCR analysis revealed enhanced expression of four α-mannosidases in pupae and adults compared to larvae. In line with this finding, we found that adult proteins contained more processed *N*-glycans than larval proteins. Moreover, silencing of genes involved in *N*-glycan processing generated diverse effects related to metamorphosis; e.g. knockdown of α-mannosidases produced malformation of wings, elytra and legs, while knockdown of α-glucosidases blocked the larval-pupal molt. Altogether, our findings provide novel evidence that *N*-glycosylation is one of the key processes in the metamorphosis of the red flour beetle, *T. castaneum*. Further investigation of insect glycobiology would grant deeper insight into the events driving insect metamorphosis and it can yield novel strategies for pest insect control.

P162 Nicole Webster, University of Alberta

Bridging pattern and process: How do snails grow shell sculpture?

Mollusc shells are a prime example of "endless forms most beautiful" and exhibit many diverse and complicated patterns, including both colour and sculpture. Shells are formed by the mantle, a flap of tissue that surrounds the opening of the shell (aperture), secreting the necessary components for biomineralization. Malacologists have catalogued these shell patterns, and have also made great strides in understanding the molecular processes involved in calcium carbonate secretion. However, nothing is known about the how the process of shell secretion can be modified to yield different patterns of shell sculpture. Here we work to answer: What aspect of the mantle change to produce different shell sculpture? Nucella ostrina is a small intertidal predatory snail with plastic shell sculpture, varying from strong spiral ribs to a smooth shell. We examined the mantle of ribbed and smooth snails using histology, 3d reconstructions, and histochemistry. We show that changes in the dimensions of the mantle epithelium and enzymatic expression patterns correlate with the placement of spiral ribs in N. ostrina.

P163 Judith Wexler**,** University of California, Davis

Do all insects share a common sexual differentiation pathway?

Male and female development in insects has been thoroughly characterized in the model organisms Drosophila melanogaster, Apis mellifera, and Tribolium castaneum. However, these species are members of a derived, monophyletic group – holometabola – which makes inference about the evolution of this developmental pathway difficult. Holometablous insects are the only animals known to use sex-specific splicing to control male and female differentiation. Sexually dimorphic splicing of the protein transformer renders the male transcript non-functional. In females, a functional transformer transcript directs female specific splicing of the transcription factor doublesex. To address when this developmental mechanism evolved, I am testing whether transformer and doublesex have sex-specific isoforms in insect species that diverged prior to the origin of holometabola. By cloning these genes from cDNA, I found conservation of sexually dimorphic spliceforms of transformer and doublesex in the cockroach Blatella germanica and

conservation of sex-specific splicing of transformer in the kissing bug Rhodnius prolixus. To test whether the function of the male and female isoforms of these genes is conserved, I am using RNAi to knock down transformer and doublesex in cockroaches (B. germanica). The transcripts I've cloned from cockroaches suggest that sex-specific splicing of transformer and doublesex may have evolved near the base of the insect class. To test this hypothesis, I'm cloning transcripts of transformer and doublesex in the mayfly Ephemera danica. These investigations into the basic biology of basal insect species will allow us to identify developmental synapomorphies of holometabolous insects and of insects as an entire class.

P164 Katherine Woronowicz, University of California, San Francisco
FGF and TGFβ Signaling Regulate Species-Specific Form and Function of the Coronoid Process in the Avian Jaw
Mechanical stimulation is essential for proper musculoskeletal development. Such integration of skeletal form and function enables morphological plasticity and species-specific adaptation. An important example is secondary cartilage that arises in certain tendons in response to mechanical forces. In humans, rats, and duck, secondary cartilage forms at the insertion of the jaw adductor muscles on the coronoid process. An equivalent secondary cartilage is absent in mice, chick, and quail. Why secondary cartilage arises at this location in some species and not others is unclear. We hypothesize that differential forces generated by species-specific jaw movements and muscle attachments lead to induction of secondary cartilage through FGF and TGFβ signaling. To test our hypothesis we investigate embryonic motility, jaw anatomy, the mechanical environment, and FGF and TGFβ signaling in quail and duck embryos. We find that quail and duck have equivalent embryonic motility but significant differences in jaw anatomy. The mandibular adductor muscle inserts dorsally in quail whereas this muscle wraps around the mandible and inserts laterally in duck. We predict that these differences produce tension at the insertion in quail, and tension plus compression in duck. Compression is a potent pro-chondrogenic stimulus and we use finite element analysis to model the mechanical environment in duck versus quail. We also find that FGF signaling is species-specific, down-regulated following paralysis, and required for induction of secondary cartilage. Likewise, inhibiting TGFβ signaling inhibits secondary chondrogenesis. Thus, we propose that species-specific jaw anatomy generates compression, which stimulates secondary chondrogenesis via an FGF and TGFβ signaling-dependent process.

P165 Jie Xiang, University of Maryland, College Park
***Dermestes maculatus*: An intermediate germ beetle model system for evo-devo**
Evo-devo studies require new model systems that represent distinct features, branches of phylogeny, and for which molecular genetic approaches are feasible. *Drosophila melanogaster* is a canonical model for studying insect segmentation, with a well-characterized cascade of regulatory genes controlling segment development and identity.

However, its highly derived long germ mode is not representative of sequential segmentation found in most insects. The *Drosophila* pair-rule genes (PRG) are responsible for formation of segments in *Drosophila*, but the expression and function of at least some of these genes have changed during insect radiations. Here we have established *Dermestes maculatus* (*Dmac*) as a new model system for comparative studies of segmentation patterning within Coleoptera. We investigated *Dmac* early embryogenesis using nuclear and phalloidin staining. To our knowledge, this is the first time the early steps of nuclear division have been characterized in this species. Orthologs of the nine *Drosophila* PRGs were isolated, and their expression was examined using RNA *in situ* hybridization. These expression patterns confirmed *Dermestes*' intermediate germ mode of segmentation and indicated that pair-rule segmentation patterning mechanisms are employed in this species. Knockdown using RNA interference revealed pair-rule function of some *Dmac* PRG orthologs. Future studies on other segmentation gene orthologs will unravel mechanisms underlying segmentation patterning in intermediate germ insects, and comparative studies will provide insight into the evolution of segmentation networks in beetles with diverse germ modes. Furthermore, the successful application of molecular genetic approaches suggests *Dermestes*' potential as a practical model for insect molecular studies.

P166 Min Ya, Harvard University

JAGGED* regulates lateral organ development and leaf adaxial identity in *Aquilegia

As the exploration of genetic frameworks of plant architectures proceeds, many key players in the fundamental morphogenesis programs have been pinpointed. First identified in Arabidopsis, *JAGGED* (*JAG*) is recognized as an essential gene in coordinating cell division and expansion during lateral organ development. To explore the degree of functional conservation of *JAG* across different species, we investigated the expression patterns and knockdown phenotypes of its ortholog in *Aquilegia coerulea*. *In situ* hybridization showed strong expressions of *AqJAG* throughout the floral meristem, all arising floral organ primordia, and regions expanding rapidly in developing flower buds. Knockdown of *AqJAG* expression through virus induced gene silencing (VIGS) resulted in laminar reductions in perianth organs and leaves; a decrease in petal and/or sepal numbers; distorted leaf margins; and curvature towards the abaxial side of the leaf lamina, forming a bowl-like structure. Scanning electron microscopy (SEM) revealed a decrease in cell sizes and numbers on both leaf surfaces and a change in adaxial cell identity in VIGS-treated leaves. Our results suggest that similar to functions of other *JAG* homologs, *AqJAG* promotes primordia initiation and lamina expansion. However, expressions of *JAG* were strictly excluded from floral meristems in all previously studied species expect for rice, indicating that *AqJAG* involves in additional meristem regulatory networks. Moreover, the abnormal margin development, dramatic lamina reduction, and alternation of adaxial cell identity in leaves are knockdown phenotypes

unique to *AqJAG*. This strengthens the possibility that compound leaf development in *Aquilegia* involves novel genetic mechanisms.

P167 Itai Yanai, Technion - Israel Institute of Technology

The phyletic-transition and the origin of animal body plans

Morphological and molecular analyses have revealed that a stage in mid-development – known as the phylotypic period – is conserved across species of the same phylum. However, the degree to which this stage may be universal to all animals has not been addressed systematically. Here, we compare the developmental transcriptomes of ten species, each of a different phylum, representing a wide range of life histories and embryonic forms. We find that animal embryonic development comprises the coupling of early and late conserved gene expression modules, with a 'phyletic-transition' module that occurs at the apparent phylotypic period of each phylum. Surprisingly, expression at the phyletic-transition is less conserved across phyla than that in either early or late modules, suggesting that transcriptional circuits and signaling mechanisms at this stage are body-plan specific. From these observations, we propose that a phylum may be defined as a collection of species whose gene expression at the phyletic-transition is both highly-conserved among them, yet highly divergent relative to more distantly-related species.

P168 Shinja Yoo, University of California, Berkeley

Spatial control of co-expressed wnt1 and wnt6 orthologs suggests the possibility of a stem cell niche composed of the A, B and C quadrant macromeres that envelope the D quadrant-derived posterior growth zone of the leech *Helobdella austinensis*

The Wnt signaling pathway is evolutionarily ancient, with 13 wnt genes in the last common ancestor of cnidarians and bilaterians. Wnt signaling functions in axial growth and patterning, processes that are also ancestral to these two clades. Leeches provide experimentally tractable embryos are good models to investigate the deployment of Wnt signaling during axial growth in Lophotrochozoa/Spiralia. In leech, stereotyped cleavages convert the D quadrant into a posterior growth zone (PGZ) composed of five bilateral pairs of lineage-restricted stem cells (teloblasts) and columns of their progeny (blast cell bandlets). The PGZ is enveloped by processes of the A, B and C quadrant macromeres. Bandlets merge at the surface into bilateral arrays (germinal bands) from which segmental mesoderm and ectoderm arise. In molecular phylogenies, wnt1 and wnt6 genes comprise sibling sub-families, and wnt1 and wnt6 genes are adjacent in genomic scaffolds of several species. Intriguingly, we find that in the leech, wnt1 and wnt6 are co-expressed in the ectodermal teloblasts and bandlets, turning off as blast cells enter the germinal bands. These genes are also expressed in supernumerary blast cells that never enter the germinal bands or contribute to segments. These observations indicate that wnt1/6 expression is driven by the presence of blast cells in the PGZ itself, rather by a blast cell-specific segmentation clock. To test this conclusion, we will observe the patterns of wnt1 and wnt6 expression

under conditions where the normal progression of blast cells from the PGZ into the germinal bands is experimentally perturbed.

P169 Jr-Kai Yu, Academia Sinica, Taiwan

Asymmetric localization of germline-related gene products during early development of amphioxus and its implications for the mosaic property of cephalochordate embryos

For multicellular animals, the segregation of germline cells from somatic cells is a critical step during their life history. In recent years, comparative studies in diverse organisms using conserved primordial germ cell (PGC) markers, such as Vasa, Nanos, Piwi/Ago and Tudor gene products, have provided interesting insights into the evolution of PGC specification mechanisms. We found that in cephalochordate amphioxus, maternal transcripts of Vasa, Nanos, Piwil1 and Tdrd7 become localized to the vegetal side during fertilization. These maternal transcripts aggregate into a compact granule and are inherited asymmetrically by one single blastomere during early development. Subsequently, this blastomere gives rise to a cluster of cells that display typical characteristics of PGCs. Thus our data suggest a preformation mechanism of PGC development in amphioxus, which is in contrast to the previous idea that cephalochordates form their PGCs by induction mechanism. More importantly, our data raise a surprising issue on the mosaic property of amphioxus embryos, which had long been considered as a model for highly regulative embryogenesis. The vegetal pole plasm, which is the putative germ plasm in amphioxus, is segregated into only one of the blastomeres after the first cleavage, suggesting that these two blastomeres are not exactly identical. Further studies on amphioxus vegetal pole plasm should give more insights into the evolution of early patterning mechanism in chordates.

P170 Eduardo Zattara, Indiana University

Innovation, conservation, and the function of embryonic head and brain patterning genes in the diversification of head morphologies in *Onthophagus* and *Tribolium* beetles

How the insect head is patterned is a long-standing question. Several studies focusing on anterior homeobox genes have increased our understanding of ventral head patterning, but the dorsal head is mostly *terra incognita*. Yet evolution of novel structures and changes in relative sizes of dorsal head regions have played critical roles in insect diversification. Furthermore, while most studies have addressed embryonic head patterning genes, virtually nothing is known about how the dorsal head is patterned during post-embryonic development. To close this gap, we used larval RNAi to investigate the roles of the embryonic head and brain patterning genes *orthodenticle* and *six3/optix* in instructing dorsal head formation in adults of the flour beetle *Tribolium castaneum* and the highly morphologically divergent horned beetles *Onthophagus taurus* and *O. sagittarius*. We show that in *Tribolium*, *otd1* and *otd1+otd2* RNAi adults present subtle gaps in ventral trunk plates but otherwise lack obvious phenotypic effects in the dorsal head. In contrast, *otd1* and *otd1+otd2* RNAi in *Onthophagus* horned beetles results in deletion of large portions of ventral trunk plates, and

spectacular changes in dorsal head structures, including massive reduction or deletion of horns, novel patterns of horn formation, and ectopic medial eye development. First data on *six3/optix* suggest different but complementary effects during adult head patterning. Collectively, our results suggest that embryonic head and brain patterning genes play crucial and previously undescribed roles in specifying the identity of medial epidermal structures during postembryonic development.

P171 Robert Zeller**,** San Diego State University
Evolutionary similarities between the peripheral nervous system of the ascidian larva and vertebrate sensory hair cells
WITHDRAWN

P172 Robert Zinna, Washington State University
Endocrine signals and their role in the evolution of trait exaggeration
Juvenile hormone (JH) has diverse functions in insects and is critical in the control of plastic phenotypes. This hormone is known to mediate the condition dependent expression of mandibles in male stag beetles. For example, large male stag beetles have higher levels of JH than small males during the prepupal period. Ectopic application of fenoxycarb (a JH analogue) to small males at the prepupal stage caused disproportionate mandible growth. In this study, we tested the hypothesis that JH mediates the condition-dependent expression of the elaborate horns of the Asian rhinoceros beetle, Trypoxylus dichotomus. The sexually dimorphic horns of this beetle are sensitive to the nutrition condition of the developing larva. Males receiving large amounts of food produce disproportionately large horns for their body size compared to males that receive restricted amounts of food. Unlike the stag beetle, we found it is the larval JH titer that correlates with body size in the rhinoceros beetle, instead of the prepupal JH titer. Ectopic application of fenoxycarb during the third larval instar significantly delayed pupation time, but had no effect on adult horn size relative to body size. In addition, application of fenoxycarb during the period of horn growth did not affect final horn size. Interestingly, knockdown of key JH-pathway genes using RNAi did affect the weapon size, despite ectopic overexpression of JH having no effect on trait growth. Our research demonstrates that different beetle lineages have evolved different mechanisms to respond to changes in nutrition.

P173 David Jacobs, UCLA
Homeodomain Gene Complement and Expression Across the Complex Life History of the Jellyfish *Aurelia*
Aurelia exhibits multiple distinct developments of tissue layers, nerves, and sensory features at different life history stages. These include sense-organ bearing motile planula and medusa phases. Thus studies of scyphozoan jellyfish, such as Aurelia, inform our understanding of the evolution of complex life histories, and neurosensory systems in a lineage sister to our own bilaterian group. In this study, we assembled a developmental transcriptome of Aurelia species 1., and in conjunction

with collaborative genome development, annotated the homeodomain repertoire, and compared it to Nematostella, Acropora, and Hydra, which collectively represent the other major clades of Cnidaria. Our results suggest that cnidarian homeodomains can be subdivided into 66 bilaterian families encompassing nine classes, providing a significant upwards revision for the homeodomain complement of the last common ancestor of cnidarians and bilaterians. Comparative analysis of gene regulation using RNA-Seq data suggests that Aurelia retains many aspects of homeodomains regulatory organization compared to flies and vertebrates. Despite a more complex lifecycle, Aurelia has fewer homeodomains than the anthozoans Nematostella and Acropora. While each cnidarian lineage exhibits a unique pattern of gene gain and loss, the larger homeodomain numbers in anthozoans involve gene family specific expansions. However, Aurelia has seven posterior HOX paralogs compared to two in Anthozoa, a situation somewhat reminiscent of the posterior Hox replications in the vertebrate and lophotrochozoan lineages. RNA-Seq documents dynamic expression of these genes across the Aurelia life cycle. Thus they may control the complex bodyplan/life-history evolution of Medusazoa.

P174 William Klitz, University of California, Berkeley

Straddling the impossible: methods of viral success

When an organism finds its life cycle dependent on switching between environments requiring differing accommodations necessary for survival and success, a single adaptive design cannot be optimal. Nonetheless, viruses are often called upon to thrive in such circumstances, lending a new perspective on solving developmental change in an evolutionary context. Here these challenges are explored for two single strand RNA positive viral groups (SS+RNA), the dengue virus and the enteroviruses. Valuable for our purposes each bear the advantages of intensive previous scientific study. We will see that at this one extreme corner of life (the SS+RNA viruses) developmental modification is played out through a distinct form of exploration of the possibilities of mutation and selection in response to environmental demands. The RNA dependent RNA polymerase, coded by a viral gene central to this strategy, is characterized by poor replication fidelity, resulting in an exceptionally high fraction of mutated genomes in each replicative cycle allowing adaptive success through the creation of a cloud of viral variants termed quasi-species. The utilization of the degenerative nature of the genetic code to allow particular multi-step adaptive changes may lie at the heart of the ability of enteroviruses and dengue viruses to span effective success across organs and phyla to secure environments allowing ongoing transmission.

P175 Jeffrey Neiman, University of Colorado, Boulder

Photo-mediated plasticity within the flavonoid pigment pathway

The flavonoid pathway is a well characterized pigment biosynthesis pathway found in plants. It produces several different classes of flavonoid compounds whose functions include pollinator signaling, thermoregulation, and photo protection. Although the regulation of this

pathway in several species has been well studied, the responsiveness of this pathway to abiotic signals is not as well investigated, particularly outside of crop species. This study investigated how the production of the flavonoids made by the upstream steps of the pathway (flavones and flavonols), and those made by the downstream steps (anthocyanins) varied with different light treatments in snapdragons (Antirrhinum majus). Compound production was measured for the response in flowers over the course of floral development in both treatments. The results showed that overall flavonoid production decreased with a decrease in the amount of light the flowers were receiving. These effects varied, however, for different classes of flavonoids. When light decreased, there was a small decrease found in the amount of flavones and flavonols, while the production of anthocyanins decreased substantially. Considering this response in the context of floral development, we also found that flowers do not strongly change the production of their pigments after they have undergone anthesis. Further studies will quantify the speed of plastic responses in anthocyanin production in flowers and other tissues with changing light environments.

P176 David Plachetzki, University of New Hampshire, Durham

A polymodal chemo/photo-receptor cell type from the Cnidaria of evolutionary significance

Taste 1 receptors (T1R) are class C G-protein-coupled receptors that mediate sweet and savory taste perception in vertebrates. Current evidence suggests that T1Rs originated in the lineage leading to gnathostomes, but are absent in all agnathan and invertebrate lineages examined to date. Here we present evidence from phylogenomic analyses of 36 holozoan whole genome sequences that orthologs of T1Rs are present in several non-bilaterian genomes, demonstrating a pre-bilaterian origin for this sensory gene family. A corollary of our finding is that T1Rs were independently lost in several lineages including those leading to protostomes, cephalocordates and agnathans. We also describe a polymodal sensory-motor neuron (PSMN) cell type that coordinates cnidocyte-firing behavior in cnidarians. We show that transcripts of T1Rs and opsins co-localize to PSMNs in the cnidarian *Hydra magnipapillata*. In addition, studies of cnidocyte discharge behavior provide emerging evidence that T1Rs and opsins play opposing roles in mediating cnidocyte discharge where T1R signaling is excitatory and opsin-mediated phototransduction is inhibitory. Our findings reorder the current view of the evolutionary history of T1Rs and suggest that this sensory gene family was in fact present prior to the major diversification events in animals, but lost in several lineages independently. In addition, the nature of cnidarian PSMN cell types, where both T1Rs and opsins contribute to function, suggests novel hypotheses for the origins of animal sensory neurons and their transduction cascades.

P177 Desmond Ramirez, University of California, Santa Barbara

Deeply conserved r-opsin phototransduction cascade genes may underlie a novel expansion response of chromatophores to light in isolated Octopus skin

Understanding the evolutionary origins of novel traits, whether morphology, physiology or behavior, sheds light on the processes that help shape the exuberant diversity we see in life. A key question is the extent to which novel behaviors depend on new underlying components or arise through evolutionary "tinkering", which may borrow and recombine existing components to produce new behaviors. Cephalopod mollusks like octopuses and squids change the color and pattern of their skin for camouflage and communication. Embedded in their skin are novel pigmented organs called chromatophores that they use to perform these astounding color-changing feats. While changes in body patterning are known to rely on eyesight, we have also found that bright white light causes the chromatophores in isolated Octopus bimaculoides skin to expand in the absence of eye or CNS input. We call this behavior Light-Activated Chromatophore Expansion or LACE. To identify potential molecular mechanisms that may underlie LACE, we found that r-opsin phototransduction genes are expressed in octopus skin, and identified peripheral sensory neurons in the skin that express r-opsin. LACE suggests that octopus skin is intrinsically sensitive to light, and that this dispersed light sense could contribute to their unique and novel camouflage abilities. Further, finding r- opsin phototransduction cascade genes expressed in octopus skin suggests that a common molecular mechanism for light detection in eyes may underlie LACE and may have thus been co-opted for light sensing in octopus skin.

P178 Dana Rashid, Montana State University

The DinoChicken Project: Development and evolution of the avian tail

The avian tail, from its maniraptoran dinosaur origins to its present-day configuration, has undergone significant alterations in morphology and function. The ancestral long tail likely served as a counterbalance for weight distribution, but the more modern short bird tail has been adapted specifically for flight. A critical junction in avian tail evolution occurred approximately 125 million years ago, at which point the tail truncated and distally fused. To study the mechanisms behind these changes, we are examining vertebrate axial elongation and termination genetics as well as chick tail development. An analysis of vertebrate tail mutants revealed that axial truncation and vertebral bone fusions are often linked, indicating that single mutations could account for more than one morphological change in the tail. Interestingly, the pleiotropic effects of a number of axial-truncating mutants mirror the traits found in the first short-tailed birds. Tail mutant analysis also uncovered several pathways and associated developmental processes critical to tail

extension and termination. To tie modern avian biology to the Cretaceous transition to short-tailed birds, ongoing studies are documenting pertinent events during chick tail development. These studies include formation of the pygostyle (the fused bony structure at the distal end of the avian tail), comparison of tail structures in birds vs long-tailed vertebrates, and the investigation of transient atavistic intervertebral discs in the pygostyle and synsacrum.

P179 John Young, Harvard Medical School

Genomic and transcriptomic sequencing of the Great Pond Snail *Lymnaea stagnalis*

The Great Pond Snail, Lymnaea stagnalis, has served as an excellent model system for the study of host-parasite interactions, predator-prey biology, and memory formation and maintenance for the past 20 years. Recently, interest in this snail has broadened because it serves as an attractive model to elucidate the developmental genetics that govern gastropod morphology including shell chirality. The increased interest in Lymnaea as a genetic system has resulted in an increased desire for genomic tools to identify and analyze genes found to be important in embryogenesis and behavior. Currently, the genomic resources for Lymnaea remain sparse and a genome sequence is not available. To address this, we have used Illumina paired-end sequencing to assemble a draft genome from a single individual. The assembly, while fragmented, allows for identification of genes and their core promoters. We have also created a de novo Lymnaea transcriptome assembly from RNA-seq data from four developmental time points, which yielded approximately 35,000 unique genes. Though many of these purportedly unique genes are potentially splicing isoforms, over 30,000 of these genes have significant expression levels. About 70% of the transcriptome identified genes are represented, albeit sometimes split across gene scaffolds, in our current genome assembly. We have used in situ hybridization to confirm the temporal expression of developmentally relevant transcripts. The results from this work provide a framework for a sequenced genome of Lymnaea stagnalis, and thereby broaden our understanding of the genomic diversity of the lophotrochozoa as well as their patterns of genetic development.

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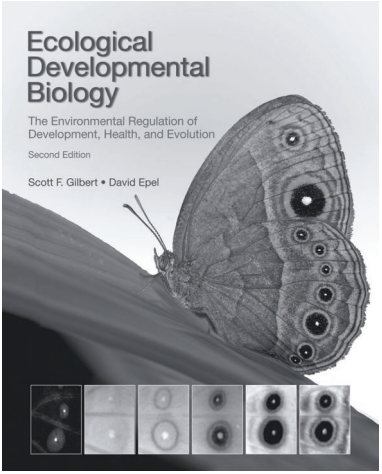

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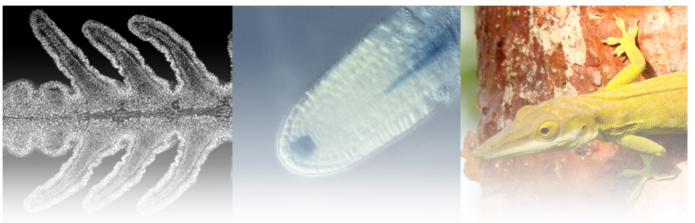

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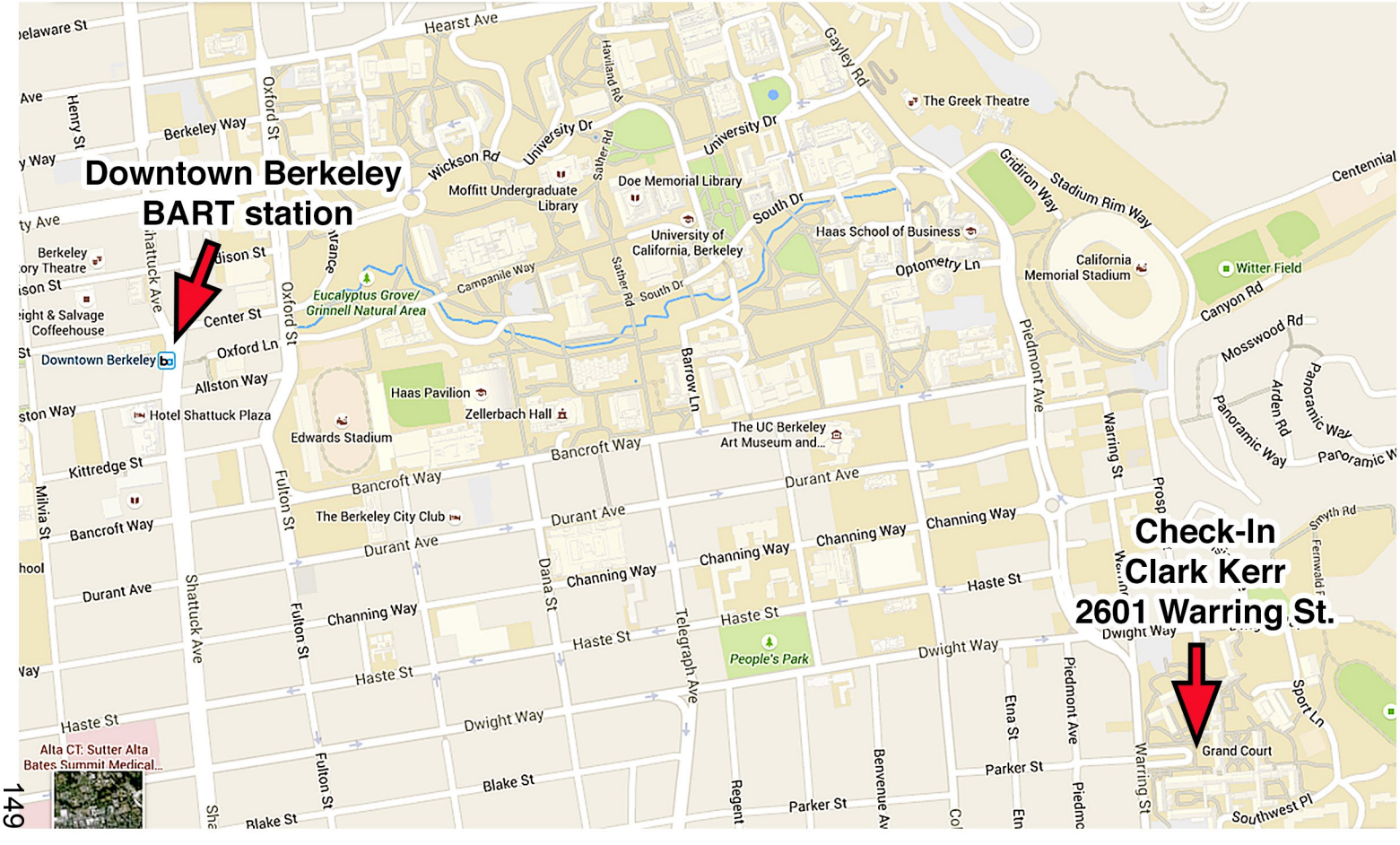
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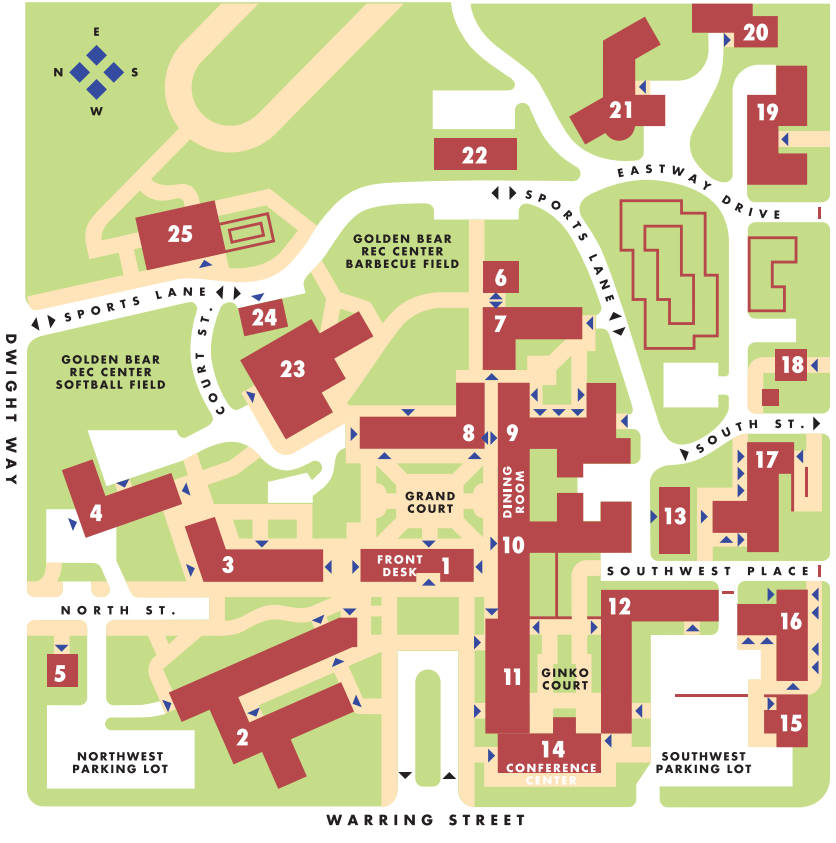

1. Administration: Front Desk, Academic Center, Conference Services Office, Conference Room
2. Suites
3. Residence Hall
4. Residence Hall
5. Child Care
6. Residence Hall
7. Residence Hall
8. Residence Hall
9. Suites
10. Dining Center, Garden Room, Great Hall, Executive Dining Room
11. Suites
12. Residence Hall
13. Steam Plant
14. Clark Kerr Campus Center, Joseph Wood Krutch Theatre Conference Rooms 102, 104, 203, and 204
15. Child Care Center
16. Suites
17. Suites
18. Faculty House
19. Faculty Apartments
20. Faculty Apartments
21. Archives
22. Archives
23. Zone 1 Physical Plant
24. Recreation Maintenance
25. Golden Bear Recreation Center

▸ Indicates Building Entrance

Meeting At A Glance

time	Wednesday (Aug 5th)	Thursday (Aug 6th)	Friday (Aug 7th)	Saturday (Aug 8th)
7:00 - 8:00 AM		Breakfast	Breakfast	Breakfast
8:00 - 8:30 AM	*8AM - 3PM*	Mark Martindale		
8:30 - 9:00 AM	*Tribolium Meeting*	Rich Palmer	Concurrent Sessions	Robb Krumlauf
9:00 - 9:30 AM	*Rm 104*	Jocelyn Hall	2 X 6 talks (15 min)	James Umen
9:30 - 10:00 AM		Veronica Hinman	see schedule below	Manu Prakash
10:00 - 10:30 AM		Coffee Break	Coffee Break	Coffee Break
10:30 - 11:00 AM		Craig Miller	Concurrent Sessions	Deniz Erezylimaz
11:00 - 11:30 AM		Chris Amemiya	2 X 6 talks (15 min)	Alexa Bely
11:30 AM - Noon		Mansi Srivastava	see schedule below	Bob Reed
Noon - 12:30 PM		Lunch	Lunch	Lunch
12:30 - 1:00 PM		Noon - 1:30 PM	Noon - 1:30 PM	Noon - 1:30 PM
1:00 - 1:30 PM				
1:30 - 2:00 PM		Posters	Tamara Franz Odendaal	Matt Rockman
2:00 - 2:30 PM	2 PM – 6 PM, Check-In		Julia Bowsher	Stacey Smith
2:30 - 3:00 PM	& Registration	(refreshments)	Posters & refreshments	Posters & refreshments
3:00 - 3:30 PM				
3:30 - 4:00 PM	*3-6 PM*		Rachel Collin	Ralf Sommer
4:00 - 4:30 PM	*Council Meeting*		Frietson Galis	Vivian Irish
4:30 - 5:00 PM	Rm 102	Mark Rebeiz	Break	Break
5:00 - 5:30 PM		Jose Javier Neto	James Hanken	Matt Gibson
5:30 - 6:00 PM	Dinner on	Catherine Linnen	Angela Hay	Poster & Talk Awards
6:00 - 6:30 PM	your own	Dinner	Dinner	Dinner
6:30 - 7:00 PM		6:00 PM - 7:30 PM	6:00 PM - 7:30 PM	5:45 PM - 7:15 PM
7:00 - 7:30 PM	Introduction			
7:30 - 8:00 PM	Keynote talks:	Workshops	Igor Schneider	Award talks
8:00 - 8:30 PM	Sean Carroll	(concurrent in	Kim Cooper	Rudy Raff
8:30 - 9:00 PM	Neelima Sinha	Krutch Theater		Natalia Pabon-Mora
9:00 - 9:30 PM	Opening Reception	and Garden Room)	Future of EvoDevo	Closing Reception
9:30 - 10:00 PM				

Concurrent session schedule (Friday Aug 7; 12 min talk + 3 min questions)

	Krutch Theater	**Garden Room**
8:30 - 8:45 AM	Andrew Gillis	Carolyn Wessinger
8:45 - 9:00 AM	Alberto Stolfi	Evangeline Ballerini
9:00 - 9:15 AM	Carlos Infante	Alma Pineyro-Nelson
9:15 - 9:30 AM	Jennifer Maier	Cecilia Zumajo Cardona
9:30 - 9:45 AM	Karen Crow	Marianna Benitez
9:45 - 10:00 AM	Ricardo Mallarino	Deirdre Lyons
Break 10:00 - 10:30 AM		
10:30 - 10:45 AM	Sylvain Marcelini	Erin Jarvis Alberstat
10:45 - 11:00 AM	Jessica Gray	Emily Delaney
11:00 - 11:15 AM	Brent Hawkins	Yi-Jyun Luo
11:15 - 11:30 AM	Yi-Hsien Su	Eric Camino
11:30 - 11:45 AM	Arnaud Martin	Pamela Windsor-Reid
11:45 AM - Noon	Paul Gonzalez	Sofia Casasa

Sunday Breakfast
7AM - 9 AM
Departure by Noon